

A NEW METHOD OF ANALYSIS BASED ON
ROOM TEMPERATURE PHOSPHORESCENCE

By

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DEDICATION

Without the love, devotion, and confidence of my wife, Susie; my mother, Lucy; my father, LaVerne; and my mother and father-in-law, Helen and William Howard, the work presented in this dissertation would not have been possible. To them goes my eternal gratitude and to them I dedicate this manuscript.

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Chairman: James Dudley Winefordner
Major Department: Chemistry

The present study indicates that room temperature phosphorescence has tremendous potential for analysis of a wide variety of ionic organic molecules and that it can be used for quantitative and qualitative identification purposes. The substances tested are representative of a wide variety of aromatic carboxylic acids, amines, thiols, or phenols. Many of the compounds are of biological importance or pharmaceutical interest.

The findings indicate that this method provides a sensitive, selective, and accurate means of identifying certain aromatic molecular species which are ionic. Although there is no strong theoretical explanation for

this phenomena, it is believed that the ionic state of the molecules results in great molecular rigidity via adsorption to the substrate, which reduces radiationless decay due to collisional deactivation.

Room temperature phosphorimetry offers many advantages as an analytical method. It has good sensitivity with the potential to be much more sensitive. It is inexpensive and safe in the sense that liquid nitrogen or cryogenic equipment is not required. It is convenient and does not require expensive chemicals or high purity solvents. This technique is a rapid method of analysis which could be automated. The procedure established is fairly simple and would be easy for a technician to learn.

Room temperature phosphorescence definitely merits future study. The technique developed should find many applications to real systems, particularly for biological and pollution samples. Studies that are of importance for future research include the investigation of better substrates, automatic sampling, the influence of pH and solvent, and the analysis of species in blood serum, urine, and air pollution particles. Finally, it would seem that time-resolved phosphorimetry should provide additional selectivity in the measurement of real samples.

CHAPTER I

INTRODUCTION

Molecular phosphorescence spectroscopy or phosphorimetry is a relatively new luminescence method for molecular analysis. It is usually applied to unsaturated organic molecules with their π -bonding arranged in a conjugated manner. Previously, intense phosphorescence has been almost exclusively observed at low temperature (less than 150°K). As a result, earlier research in phosphorimetry as a method of analysis has been performed at low temperatures, principally in liquid nitrogen because this liquid is the most convenient to use.

The existence of phosphorescence has been known for a number of years since it was first reported in 1888 by Wiedemann (1). Schmidt (2) in 1896 observed the increase of phosphorescence intensity when measured at low temperatures. It was first suggested as a method of analysis by Lewis and Kasha in 1944 (3). Its utility as a method was not realized until publication of a study by Keirs, Britt, and Wentworth in 1957 (4). In the last ten years, its use as an analytical technique has undergone phenomenal growth. Applications of phosphorescence to routine analytical problems may be found in a variety of areas: coal tar

fractions, antimetabolites, and carcinogenic hydrocarbons, to name a few (5, 6, 7).

As a method of analysis, phosphorescence compares favorably with competing electronic spectroscopic techniques, mainly molecular fluorescence and ultraviolet absorption. The standard criteria used for comparison of analytical spectroscopic methods are i) breadth of application, ii) selectivity, iii) sensitivity, and iv) accuracy and precision.

(i) Most unsaturated organic compounds show measurable ultraviolet absorption, but not all of them re-emit the absorbed radiation as measurable fluorescence or phosphorescence. Therefore, absorption spectroscopy has a greater breadth of application than either fluorimetry or phosphorimetry. However, all three methods are suitable for many compounds for qualitative and quantitative determination. Thus, the selection of the method which should be preferred can be decided on the spectroscopic characteristics of the system of interest.

(ii) In regard to selectivity, phosphorimetry is the superior method of analysis. Just as in fluorimetry, the two spectra obtained, excitation and emission, can be utilized for identification. The

phosphorescence spectra are usually more characteristic than the fluorescence spectra and are therefore frequently better suited for identifying compounds. Because of the wide range of measurable lifetimes of phosphors, time and phase resolution can also be used for identification and separation of complex mixtures - methods which are not possible in absorption spectrometry and which are instrumentally difficult in fluorimetry (8).

- (iii) Luminescence methods, in general, result in ten to a thousand times lower detection limits and greater sensitivity than absorption techniques. Because the scatter of source radiation in phosphorescence is much lower than in fluorimetry, wider monochromator slits and apertures can be used. Relative limits of detection determined in fluorimetry and phosphorimetry vary widely from compound to compound depending on whether noise levels, absorption coefficients, and quantum yields are favorable. McGlynn in 1963 (9) on the basis of three compounds, and in 1966, Sauerland and Zander (10) on a wider basis, concluded that spectrophosphorimetry and spectrofluorimetry had comparable sensitivity. Since

then, Winefordner and co-authors have greatly improved the sensitivity of compounds measured by phosphorimetry. The use of a rotating sample cell, more stable source, open ended capillary, and lower solvent background has increased sensitivity by several orders of magnitude (8), making phosphorimetry the most sensitive technique.

- (iv) All three spectrometric techniques have comparable accuracy and precision. For example, the relative standard deviation of phosphorescent measurements is less than 1% using the rotating sample cell with 10^{-5} molar solutions (8).

Thus, phosphorimetry with samples maintained at low temperatures is now an important and established technique for trace analysis. The major disadvantage of phosphorimetry is the rather inconvenient need of low sample temperatures. The need for such low temperature requirements results in greater expense and complexity of analyses due primarily to the added cost and use of liquid nitrogen or other coolants. Also, such low temperatures make the automation of phosphorescent analysis virtually impossible. Therefore, if phosphorimetry could be carried out at higher temperatures with the same sensitivity and selectivity advantages, then it might find greater use in real applications.

Phosphorescence has been observed at room temperature, but at that temperature it is usually very weak and of little analytical interest. For example, phosphorescence at room temperatures has been reported in gaseous biacetyl vapor (11), in liquid biacetyl solutions (12), in solid boric acid or sugar solutions (13), or in very rigid plastics such as polymethyl methacrylate (14). None of these matrices are suitable for quantitative analyses. They are either too specialized as in the case of biacetyl or too difficult and time consuming as in the other cases. Polymer solutions also are subject to interferences from their own ultraviolet absorption and phosphorescence emission.

In 1972, Schulman and Walling observed intense phosphorescence of several molecules at room temperatures (15, 16). They reported that when certain organic substances were ionically adsorbed on silica gel, alumina, paper, or asbestos and thoroughly dried, efficient triplet state emission occurred. These studies dealt only with some of the qualitative aspects and no attempt was made to develop analytical applications of this phenomenon.

Thus, this dissertation will deal principally with the development and evaluation of a method of analysis using room temperature phosphorescence of ionic organic molecules adsorbed on a substrate, primarily filter paper. This dissertation will demonstrate that room temperature phosphorescence

offers a fast, economical, and convenient method of analyzing a variety of organic molecules, many of pharmaceutical or biological importance.

CHAPTER II

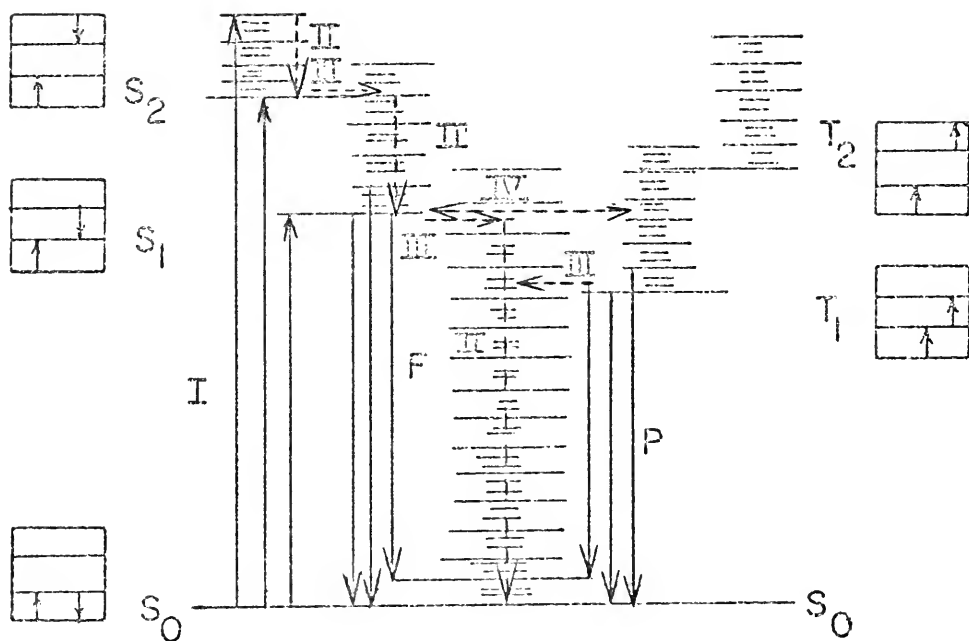
THEORETICAL CONSIDERATIONS OF PHOSPHORESCENCE

In 1935, Jablonski (17) was the first to discuss the theoretical basis of phosphorescence. Later, Lewis and Kasha (3, 18) firmly established the theory explaining this phenomenon. Since that time, experimental measurement and calculation have thoroughly verified their work. Their discussion of the theory pertinent to this manuscript will be restricted to the luminescence phenomena of an organic species in solution. Other sources of phosphorescence, such as from physical defects in crystals, will not be discussed here.

Consider a liquid solution of an organic compound that exhibits strong ultraviolet or visible absorption. If that solution is irradiated with electromagnetic radiation of the wavelength of the absorption, then it will emit isotropic radiation of a longer wavelength at room temperature. This is commonly known as fluorescence. Next, suppose that the solution is cooled to low temperatures or somehow becomes a rigid matrix. If this solid solution is again subjected to ultraviolet or visible radiation of the species' absorbing wavelength, it will

again fluoresce isotropically as well as emit radiation of a wavelength longer than the fluorescence; this latter emission will have a lifetime that is several orders of magnitude longer than the lifetime of fluorescence. The long-lived phenomenon is called phosphorescence; this radiation will generally be in the near ultraviolet or visible region of the electromagnetic spectrum (between 350 nm to 600 nm) and the lifetime of phosphorescence will be of the order of milliseconds to seconds. Phosphorescence decay follows first order kinetics.

Fluorescence and phosphorescence processes will be considered for a single unsaturated organic molecule. Figure 1 is a modified Jablonski diagram showing some of the energy levels of a typical unsaturated organic molecule. For the moment, consider only the electronic energy levels of a molecule with a pair of electrons in the ground state, denoted as S_0 . Each electron is given a spin angular momentum quantum number of either $+\frac{1}{2}$ or $-\frac{1}{2}$. In Figure 1, electrons having a $+\frac{1}{2}$ value are shown as arrows pointed upwards, and those of $-\frac{1}{2}$ are pointed downward. The Pauli exclusion principle requires two electrons in the same orbital to have opposite spins, as shown in the box next to S_0 in Figure 1.



Symbol	Process	Rate Symbol	Range of Rate Constants, sec ⁻¹ (9)
I	Absorption	k _I	10 ¹⁵ - 10 ¹⁶
II	Internal Conversion	k _{II}	>10 ¹²
III	Vibrational Relaxation	k _{III}	>10 ¹²
IV	Intersystem Crossing	k _{IV}	10 ⁴ - 10 ¹²
F	Fluorescence	k _F	10 ⁶ - 10 ⁹
P	Phosphorescence	k _P	10 ⁻² - 10 ⁴

Fig.1.--Electronic transitions resulting from the absorption of a photon. Absorption and emission of photons is indicated by straight lines. Nonradiative transitions are indicated by broken lines.

If a photon of the appropriate energy is absorbed by the molecule, then one of these electrons may be promoted to a higher energy level. The promotion process may occur in one of two manners. In the first case, the electron's spin angular momentum quantum number is not changed. Thus, the spin multiplicity, defined as one plus twice the absolute value of the sum of the spin angular momentum quantum numbers of the electrons, will be unity. States having a spin multiplicity of one are called singlet states (S). Note that the multiplicity of the ground and excited states in this case will be the same. In the other case, the spin of the promoted electron changes. Because an electron's spin quantum number may have only two values, the promoted electron now has the spin quantum number the same as the one remaining in the ground state. The multiplicity of this condition is three and called a triplet state (T). It follows from Hund's Rule that the first triplet state (T_1) is of lower energy than the first excited singlet state (S_1).

However, usually only singlet-singlet ($S_0 \rightarrow S_1$) promotions are observed. This can be explained from the first order quantum mechanical treatment of electron

states; transitions are allowed only between states of the same multiplicity. This is only an approximation and singlet-to-triplet absorption is observed, although very weakly, i.e. as Sklar reported in the absorption spectrum of benzene in 1937 (19). In this case, the $S_0 \rightarrow S_1$ absorption is about 10^5 times more intense than the $S_0 \rightarrow T_1$ absorption. Because the latter absorption will not be pertinent to the work described in the manuscript, it will not be considered further.

Fortunately, there are other more efficient ways of populating the triplet state than direct radiational excitation. Those molecules excited to one of their excited singlet states can sometimes be partially de-excited to the triplet state via several different processes. Consider a molecule that is in one of its higher excited singlet states (S_2, S_3, \dots). It may reach a lower state of the same multiplicity through a crossing point of their potential energy curves. This process is called internal conversion and is indicated as process II in Figure 1. In the absence of intermolecular collisions, internal conversion is restricted to excited electronic states because the energy differences between individual excited electronic states are much smaller than between ground and excited states, and so vibronic

coupling between the first excited electronic state and the ground state is much less likely to occur, preventing complete radiationless deactivation.

The excess energy resulting from the vibrational relaxation and internal conversion is dissipated through vibrational motions of less rigid parts of the molecules. Internal conversion and vibrational relaxation are extremely rapid processes (rate constants of ca. 10^{12} sec^{-1}); as a result, most molecules that are photon excited to high electronic states, return nonradiationally to the lowest vibrational levels in the lowest excited electronic state.

Once in the lowest excited singlet level, the molecule can deactivate itself completely by emitting a photon. This singlet-singlet ($S_0 \leftarrow S_1$) emission process is known as fluorescence (process F in Figure 1). The molecule may also be nonradiationally deactivated through intermolecular collisions, or quenching. A third alternative is that the electron in the excited singlet state may "cross over" to the next lowest triplet state. This process is called intersystem crossing (process IV in Figure 1). The rate constant for this process is about 10^8 sec^{-1} but varies over a wide range depending on (i)

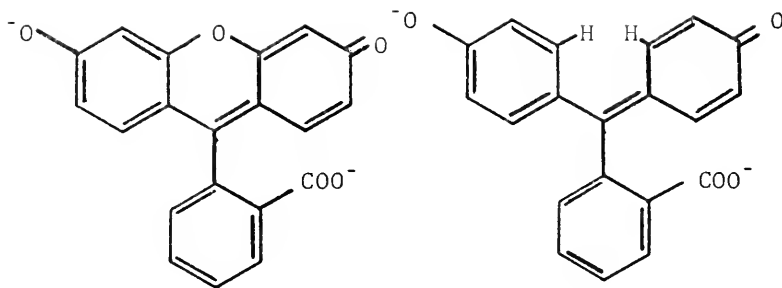
the energy difference between the singlet and triplet systems and (ii) on the degree of mixing of singlet character in the triplet state. Again, first order quantum mechanics forbids transitions between states of different multiplicities. But this is not true if there is significant spin-orbit coupling or mixing of the singlet and triplet levels. Kasha in 1950 measured the rates for intersystem crossing in ($\pi \rightarrow \pi^*$) systems and found them to be about 10^7 sec^{-1} (20) which is about 10^5 times slower than vibrational or internal conversion; thus, intersystem crossing will occur almost exclusively at the lowest excited singlet and triplet states.

The triplet state may be deactivated in several ways. First, the electron may undergo reverse intersystem "cross-over" to the singlet system. If the molecule is then radiationally deactivated, then the emission is referred to as delayed fluorescence. The process occurs in the dye eosin and is also known as E-type fluorescence. Also, the triplet state of the molecule may be nonradiationally deactivated or quenched. Typical quenchers are solvent molecules or impurities, e.g., dissolved oxygen. A third and desired possibility

is that the triplet state will deactivate emitting a photon. This process is called phosphorescence and is indicated in Figure 1 as process P.

Several important conclusions can be drawn from the varying orders of the rate constants. Phosphorescence and fluorescence emission spectra will usually be single-banded because k_f and k_p are so much smaller than k_{II} or k_{III} . Kasha's rule specifies that only the prevailing lowest state of a given multiplicity of any molecule is capable of emission.

The structure of luminescent-type compounds may have a large effect on the kinetic processes involved in the deactivation of the excited species. Straight chains or non-rigid aromatic molecules generally show poor luminescence properties. These types of compounds are particularly susceptible to vibrational relaxation processes, and easily dissipate their electronic energy externally without emission. An example of the importance of rigidity is given in the comparison of fluorescein and phenolphthalein.



fluorescein

phenolphthalein

These compounds are identical in structural geometry except for the central ring. Because phenolphthalein has two hydrogen atoms instead of the rigidly held oxygen atom, molecular twisting is much easier than in fluorescein. Fluorescein emits a very intense luminescence radiation in liquid solution, whereas phenolphthalein emits only very weakly (21).

Temperature and viscosity effects on luminescence process are also very important. With few exceptions, strongly absorbing substances in low viscosity solutions have not been observed to phosphoresce at room temperature because the quenching of the excited molecules by intermolecular collisions occurs within the lifetime of the

excited state (about 10^{-7} sec).

In 1966, McCarthy thoroughly examined the effect on phosphorescence in solution as a function of temperature (22). He concluded that values near unity for the phosphorescence quantum yield would never be obtained at even moderate solution temperatures unless solvents of very high viscosity near 273°K were readily available. However, McCarthy noted that such a development would be of considerable analytical importance. A very important conclusion that one must draw is that phosphorimetry requires a rigid matrix. While Schulman and Walling did not discover a high viscosity solvent, they did observe a very practical method of achieving rigidity at room temperatures.

CHAPTER III

EXPERIMENTAL CONSIDERATIONS

Instrumentation

An Aminco-Bowman spectrophotofluorimeter (Silver Spring, Maryland 20910) with a Aminco-Keirs rotating can phosphoroscope attachment was used for all measurements. The excitation and emission motor-driven scanning monochromators each contained a grating ruled 600 grooves per mm and blazed at 300 nm and 500 nm respectively. Their spectral band passes were approximately 6 nm per mm slit width. A Hanovia 901-C-11 50 W xenon lamp with the Aminco ellipsoidal source condensing system was used as a continuous source. A relatively steady lamp intensity was provided by an Aminco 422-818 D.C. power supply delivering about 7.5 A to the xenon arc lamp. An encapsulated RCA 1P21 photomultiplier tube of spectral response type S-4 (300 nm to 600 nm) served as the detector (23). The photomultiplier tube was continuously maintained at 700 V, 10 mA maximum, by a Keithley 244 high voltage supply (Cleveland, Ohio 44139). Phototube signals were amplified with a low noise nanoammeter designed by O'Haver and Winefordner (24). Spectra

were recorded on an Aminco 1620-827 X-Y recorder with wavelength scan speeds of about 2 nm/sec with a nanoammeter time constant of 0.5 sec. The lamp intensity was monitored with a J.E.M. Powermaster CdS-902 photo cell (Pioneer Electronics and Research Corporation, Forest Park, Illinois 60130) at 500 nm and displayed on a Keithley 220 d.c. vacuum tube voltmeter. The monochromator wavelengths were calibrated with a mercury pen light using the technique described by Udenfriend (25).

Temperature and relative humidity (R.H.) measurements were made with a Taylor, Mason's Form, hygrometer (Scientific Products, Evanston, Illinois 60204) using wet- and dry-bulb thermometers, 2°F per division. Also, a Serdex recording hygrothermometer (Bacharach Industrial Instrument Co., Pittsburgh, Pennsylvania 15230) was used; it had a bimetal strip, 2°F per division, and a tissue membrane 2% R.H. per division. Both instruments had an accuracy of \pm 3% R.H.

Chemicals and Reagents

The analyte samples were obtained from the sources listed in the Appendix and were used without further purification.

Solvent solutions of acid and base were prepared

from distilled, filtered, and deionized water. No detectable phosphorescence background was found from these solvents whether at room temperatures or at liquid nitrogen temperatures.

All glassware was prepared by soaking for more than 24 hours and then by washing in a solution of Lakeseal laboratory glass cleaner (Peck's Products Co., Ithaca, New York 14850). After rinsing, the glassware was submerged overnight in 5-6 N nitric acid. This was followed by rinsing thoroughly more than six times with distilled water and by drying in an oven at 110°-120°C. Until use, the clean glassware was stored in dust-proof containers.

Procedure

Because no other reports of this phenomenon as an analytical tool have been made, great care was taken in developing a reproducible method of preparing and testing the samples. Although Schulman and Walling observed room temperature phosphorescence on a variety of substrates, silica, alumina, paper, asbestos, and (more weakly) glass fibers, they reported that filter paper seemed to give the best results (16).

Choice of Substrate. As a result, the selection of a rigid support for analytical determinations was restricted mainly to commercially available filter papers. Fifteen different kinds were tested for their phosphorescence background and the results are given in Table I. Eaton Dikeman 613 yielded the lowest phosphorescence signal and was used in all further studies.

A high grade chromatography paper and Metrical millipore filters (Gelman Instrument Company, Ann Arbor, Michigan 48106) were also tested. The Metrical filters lacked structural stability when treated with 1M NaOH or 1M HCl and were not considered further. The phosphorescence characteristics of the chromatography paper are also given in Table I.

Choice of Solvent. The selection of 1M NaOH as the principal solvent was made for several reasons. Earlier work on polynuclear acids by Schulman and Walling (15, 16) showed that excess aqueous NaOH resulted in the strongest phosphorescence signals. Because most of the compounds selected for study included a variety of acids and bases, many with amphoteric characteristics, other solvents were considered. However, the study of several compounds in different solvents indicates no preference of acid or base.

TABLE 1. Phosphorescence Characteristics of Filter Paper

Company ^a -Type	Excitation ^b (nm)	Emission ^b (nm)	Relative Phosphorescence Signals ^c
Curtin - 7822-A ^d	305	500	1.8
Curtin - 7760	300(316,460)	494	3.0
E-D - 613	294(320)	487	1.0
E-D - 615	316	499	4.8
Reeve Angel-201	317	517	5.0
Reeve-Angel-202	310	505	1.5
S+S - 604	292	490	3.0
S-P - F-2402	295	485	1.8
S-P - F-2406	290	485	3.0
Whatman - 1	290	491	2.6
Whatman - 30	294(316)	490	5.0
Whatman - 40	294(320)	480	2.0
Whatman - 41	294(316)	486	3.5
Whatman - 42	300(322)	495	1.6
Will - 13021	295(320,390)	494	10.
Will - 13061	316	501	11.

^aKey: Curtin = Curtin Scientific
 E-D = Eaton Dikeman
 S+S = Schleicher and Schuell
 S-P = Scientific Products
 Will = Will Scientific

^bExcitation and emission peak wavelengths. Excitation wavelengths in () are shoulders.

^cAll signals taken with respect to E-D - 613 (set equal to unity), the filter paper used in the present studies.

^dChromatography paper, grade #1.

For example, a 5 mM solution of 2-amino-6-methyl-mercapto-purine dissolved in 1M NaOH, HCl, and deionized water gave equivalent phosphorescence signals. Moreover, solutions of 1M HCl weakened the filter paper and the filter paper circles were difficult to handle without disintegration. Also, almost all of the compounds to be studied were readily soluble in 1M NaOH.

Sample Preparation. Circles ($7\frac{1}{2}$ cm diameter) of Eaton-Dikeman 615 filter paper were cut into $\frac{1}{4}$ in circles using a conventional paper hole puncher. The paper circles were suspended by their edges vertically with small Micro-gator clips (Mueller Electric Co., Cleveland, Ohio 44114) in a clothesline fashion. The sample solution was allowed to run off the tip of the needle of a 50 μ l Hamilton syringe (Whittier, California 90607) until it came onto contact with the filter paper circle; 5 μ l of sample was placed on each filter paper circle. Five was chosen because it was a convenient quantity that gave even and reproducible distribution of the solution.

Methods of Drying. Drying of the samples was essential; previously reported by both Schulman and Walling (15, 16) and further shown in this laboratory that moisture on the paper sharply decreased the phosphorescence

signal. Drying initially was accomplished by 1) air drying for at least 1 hr and 2) then placing the circles in a desiccator for at least 80 min for final drying. However, this procedure took more time (total time - at least 140 min) and involved more sample handling than was felt desirable. Hot air blowers and other methods of drying, including ovens, were tested but most of these proved to be too destructive to the paper circle or the sample. Infrared lamps were gentle and very effective. The temperature of the drying area was controlled by varying the distance between the lamps and the samples. The final arrangement is shown in Figure 2. In order to reduce fluctuations of the temperature from air currents, the drying apparatus was set up in an isolated corner of the room. Two large sheets of fiber board were placed on the third and fourth sides of the drying area. This simple method worked surprisingly well. The phosphorescence of a sample could be measured in as little as 15 min after spotting. The sample needed to be handled only once, and its intensity would be equivalent to samples dried by the first method. Figure 3 is a typical graph showing the effect of drying time on phosphorescence intensity.

Fig. 2.--Drying apparatus used in room temperature phosphorescence studies. Dimensions are in cm.

Sides 1 & 2 - corner of room

Sides 3 & 4 - fiber board air shield

A - 150 watt Westinghouse Infrared Sun Ray lamps

B - Micro-gator Clip Clothesline

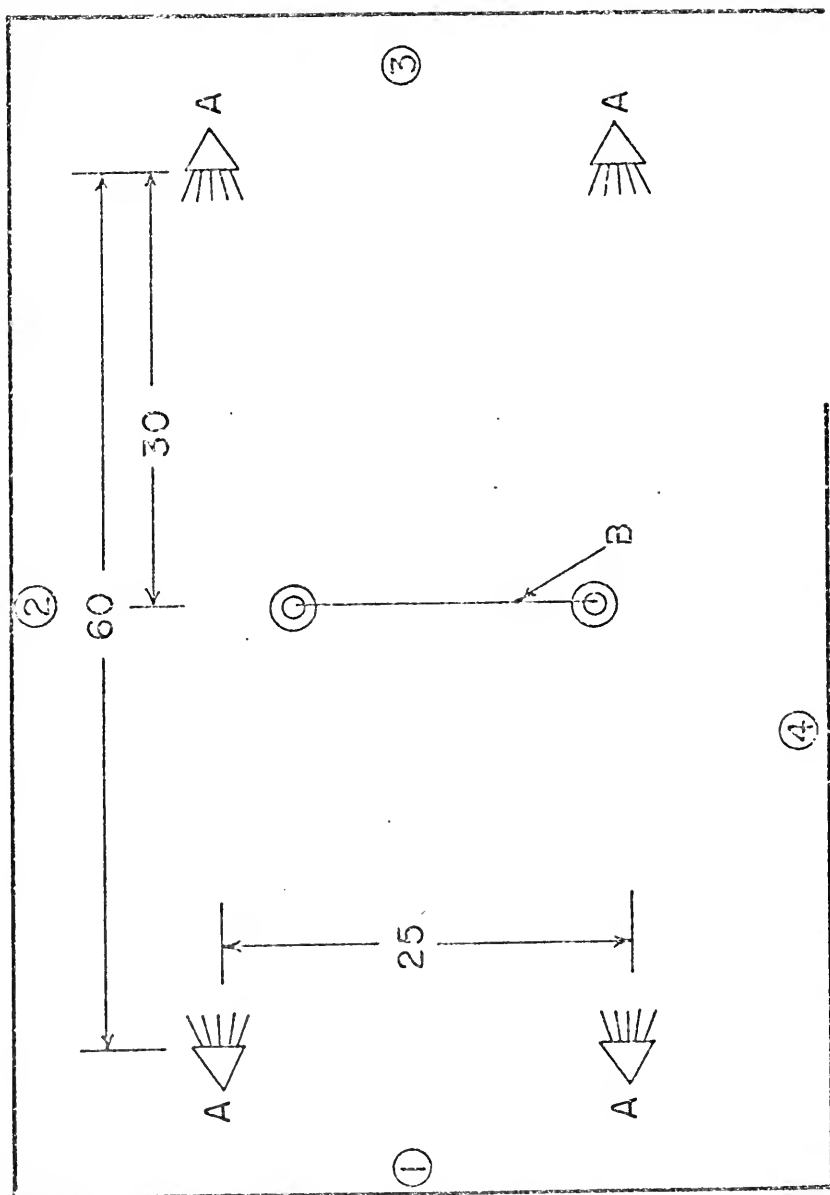
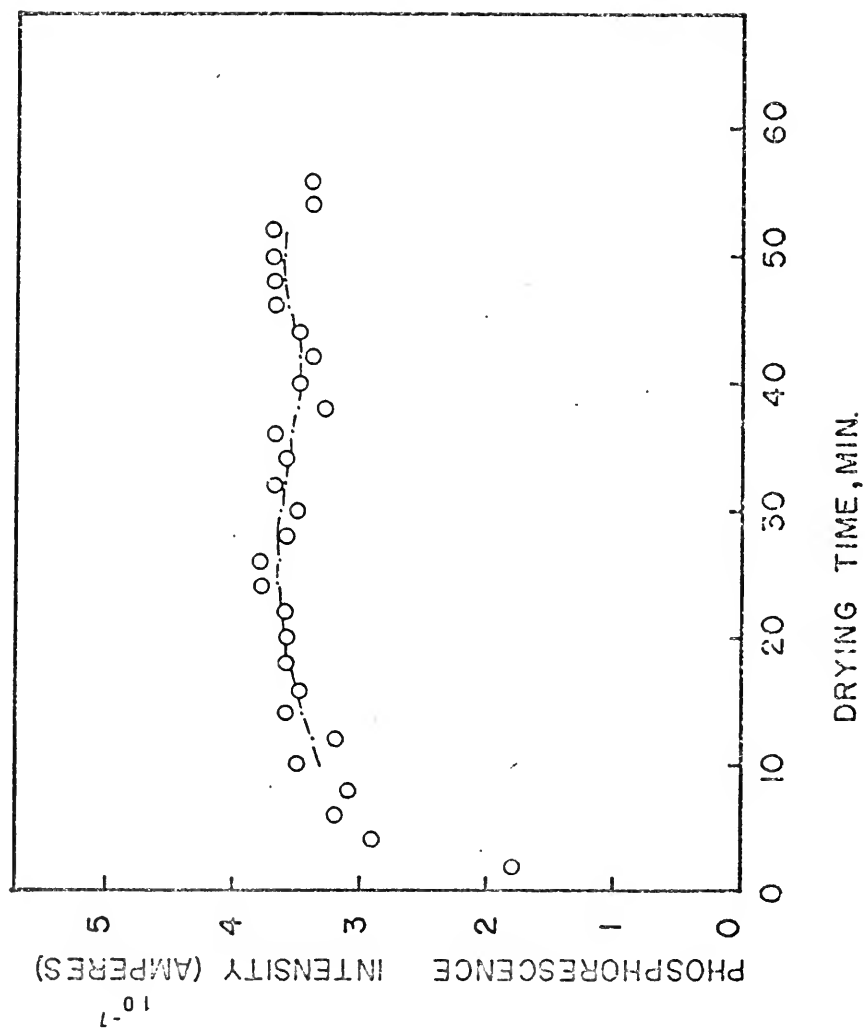


Fig.3.--Effect of drying time on intensity of
room temperature phosphorescence signal
(5mM 6-methylmercapto purine in 1M NaOH).

0 individual points

----- 5 point moving average



The relative humidity and temperature in the region occupied by the drying rack was measured at numerous points. Temperature, measured with two thermometers and the Serdex hygrothermograph, was found to be $60^{\circ} \pm 3^{\circ}\text{C}$. The relative humidity, measured with a Taylor hygrometer and the Serdex hygrothermograph, was determined to be less than 5%.

New Design of Sample Cell Assembly. In order to measure the phosphorescence of the filter paper circles in the Aminco-Bowman spectrophotofluorimeter, a number of changes in the sample cell were necessary. The rod designed to hold the samples is shown in Figure 4. Initially a strip of Scotch tape with a $\frac{1}{4}$ in hole was used to hold the paper circles. A more rigid brass plate had a hole drilled $\frac{1}{64}$ in smaller than a paper circle. This held the sample in place and gave maximal illumination. The rod, B, and cylinder C, were adjusted to a depth and angle that gave maximum phosphorescence, then tightened and sealed.

So that the sample would not absorb moisture during measurement, the sample cell was flushed with warm dry air at a rate of 15 lmin^{-1} . The volume surrounding the

Fig. 4.--Schematic diagram of sample holder for filter paper circles used in room temperature phosphorescence studies. Boxed area (dashed lines) is expanded for finer detail in Fig. 5. Dimensions are in inches and are to scale unless otherwise noted.

- A - Threaded Brass Bushings to hold B and to prevent stray light from entering sample cell
- B - Threaded Stainless Steel Rod
- C - Brass Cylinder
- D - Flat Brass Plate used to hold filter paper circle

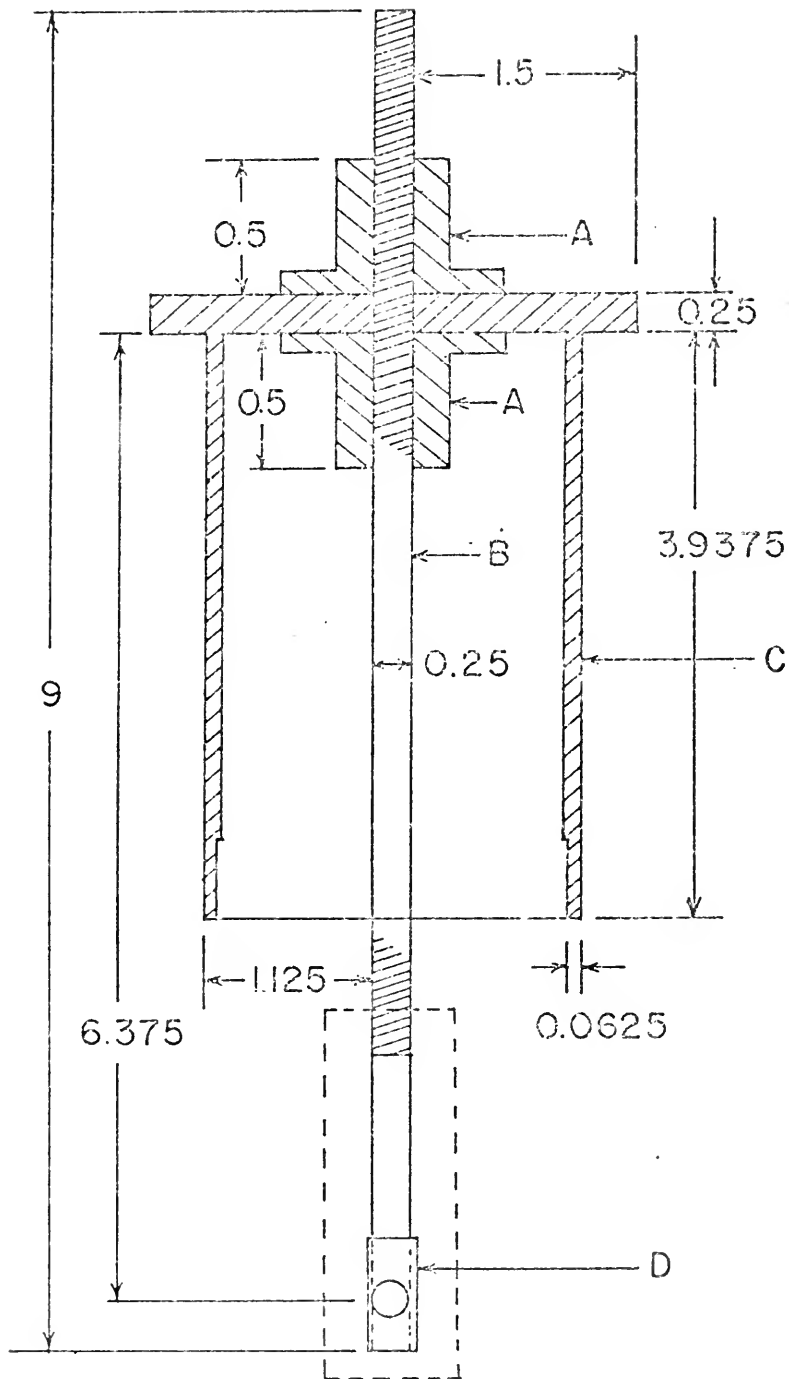


Fig. 5.--Detailed schematic diagram of boxed area in Fig. 4 of room temperature phosphorescence sample holder, designed specifically to handle $\frac{1}{4}$ in. filter paper circles. All dimensions are to scale unless otherwise stated.

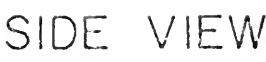
Scale: 3:1 in

B - Threaded Stainless Steel Rod

D - Flat Brass Plate

E - Filter Paper Circle

F - Flat Brass Spacers



sample cell with the sample holder in place was estimated to be about 0.5 l. The air was filtered and dried with silica gel in a drying tube and was heated to 65°C in a 18 in length of copper pipe ($\frac{1}{4}$ in outside diameter) wrapped with a Bristheat heating tape prior to entering the sample cell assembly.

CHAPTER IV

RESULTS AND DISCUSSION

General Comments

A wide variety of vitamins, purines, pyrimidines, catecholamines, and sulfa drugs were selected for study in 1M NaOH on filter paper. The purpose of this particular selection of compounds was to determine if an improved method of analysis could be developed for biological or pharmaceutical applications. Also selected were several compounds for which low temperature phosphorescence had not been observed. These would indicate whether the breadth of application of room temperature phosphorimetry could be extended beyond that of conventional low temperature phosphorimetry. Included in this selection were a number of compounds which had been studied previously in this laboratory, enabling comparison with low temperature studies under similar experimental conditions.

Those compounds that did not emit measurable phosphorescence when dissolved in 1M NaOH and dried on filter

paper are listed in Table 2.

Weakly Phosphorescent Compounds

Several of the compounds, certain purines or pyrimidines, were weakly phosphorescent. These are listed in Table 3 with their spectral characteristics and signal-to-noise ratios. Further study on these compounds was not considered analytically useful at the present time.

Intensely Phosphorescent Compounds

About one-quarter of the molecules studied emitted intense phosphorescence at room temperature using the procedure previously described. A summary of their analytical figures of merit is given in Table 4. For comparison and completeness, Table 5 shows the earlier work carried out in this laboratory by room temperature phosphorimetry using desiccator drying techniques (26). Figures 6 through Figure 15 show the quality of analytical measurements using this technique. In all cases, phosphorescence measurements were taken within at least two orders of concentration above the calculated limit of detection. The slope of the linear portion of each

TABLE 2

List of Compounds That Did Not Emit Phosphorescence
at Room Temperature in 1M NaOH on Filter Paper.

adenosine	2-amino-pyrimidine
cytidine	2,4-dichloro-pyrimidine
guanosine	2-amino-4-methyl-pyrimidine
thymine	2-amino-4,6-dihydroxyl-pyrimidine
thymidine	2-amino-4-carboxylic-5-chloropyrimidin
uracil	4,6-dihydroxyl-pyrimidine
uridine	vitamin B ₁ (thiamine hydrochloride)
folic acid	vitamin C (ascorbic acid)
riboflavin	vitamin D (calciferol)
niacinamide	vitamin K ₁
inosital	vitamin K ₃
epinephrine	vitamin K ₅
norepinephrine	vitamin H (Biotin)
tyrosine	pyridoxime hydrochloride
6-phenyl-amino-purine	sulfathiazole
6-bromo-purine	sulfamerazine
5-amino-uracil	sulfamethozine
6-amino-uracil	sulfadiazine

TABLE 3. Spectra Characteristics of Compounds That Were Weakly Phosphorescent at Room Temperature^a

Name	Excitation Wavelength ^b	Emission Wavelength ^b	Signal/Noise ^c
adenine	300	469	40
cytosine	301	405	30
guanine	282	427	40
4,5-diamino-uracil	357	524	20
2-thio-4,6-dioxy-pyrimidine	342(300) ^d	467	70

^aRoom temperature phosphorescence of these compounds is not considered at present time to be analytically useful.

^bExcitation and Emission wavelengths are accurate to ± 5 nm.

^cMeasurements made on a 5mM solution in 1M NaOH. Signal-to-noise calculated on basis of analyte intensity less blank divided by 5% of blank intensity.

^dshoulder

TABLE 4. Room Temperature Phosphorescence Characteristics of Several Ionic Organic Adsorbed on Filter Paper Using Infrared Drying^a

Compound	Ex λ , nm	b, c Em λ , nm	Slope of Linear Portion	Linear Correlation Coefficient	Limit of Detection molar	ppm	Detection range	Linear Dynamic Range
4-amino- benzoic acid	273	426	0.99	0.999	1.5×10^{-7}	0.02	0.1	10,000
2,4-dithio- pyrimidine	374	464	1.01	0.995	1×10^{-5}	1.5	7	400
4-amino-2,6- dihydroxyl- pyrimidine	310	421	0.97	0.9995	2×10^{-5}	2.	10	400
2-amino-6- methylmer- capto- purine	331	487	0.92	0.99998	5×10^{-6}	0.9	4.	300
2,6-diamino- purine	286	451	0.71	0.9994	2×10^{-6}	0.4	2	1000
6-methyl- purine	268	449	0.78	0.998	8×10^{-6}	1.0	5	100
6-chloro- purine	280	463	0.80	0.99	3×10^{-5}	3	15	50

TABLE 4. (Continued)

Compound	Ex λ , ^{b,c} nm	Em λ , ^{b,c} nm	Slope of Linear Portion	Linear Correlation Coefficient	Limit of Detection molar	ppm	Linear Dynamic Range
6-methyl- mercaptopurine	292	466	0.88	0.999	3×10^{-7}	0.06	0.3 2000
sulfanil- amide	267	426	0.81	0.99	3×10^{-6}	0.7	3. 350
sulfquan- idine	267	426	0.80	0.996	8×10^{-7}	0.2	0.9 600
trypto- phane	280	448	0.79	0.992	4×10^{-6}	0.8	4 600
5-acetyl- uracil	304	421	1.03	0.9993	2×10^{-6}	0.3	1.5 400
2-thio- 6-amino- uracil	307	441	0.88	0.999	7×10^{-7}	0.1	0.5 4000
4-hydroxyl- 3-methyloxy benzaldehyde (vanillin)	332	519	0.57	0.997	8×10^{-7}	0.1	0.6 2000

TABLE 4. (Continued)

^aAll molecules dissolved in 1M NaOH.

^bEx λ = Excitation peak; Em λ = Emission peak.

^cAll wavelengths calibrated ± 2 nm.

^dLimit of detection is the concentration of analyte resulting in a signal to noise ratio of 2.

^eLimit of detection (in ng.) is calculated from a concentrational value by using the sample volume of 5 μ l.

^fRange of linearity extends from detection limit to upper concentration where deviation from linearity is 1%.

TABLE 5. Room Temperature Phosphorescence Characteristics of Several Ionic Organic Molecules Adsorbed on Filter Paper using Desiccator Drying^a

Molecule	Excitation Peak (nm)	Emission Peak (nm)	Range of Linearity	Limit of Detection ^c (µg/ml)	Limit of Detection ^d (ng)
2-amino-1-naphthalene sulfonic acid	352	526	420	0.4	2.
eosin Y	523	569 ^e	3,000	0.02	0.1
naphthalic anhydride	285	543	1,000	0.6	3.
2-naphthlene sulfonic acid	286	511	5,000	0.2	1.
diphenic acid	277	491	10	100.	500.
naphthanic acid, sodium salt	333	541	14	17.	73.
naphthalene-2-sulfonic acid	286	518	15	28.	140.
1-naphthalene sulfonic acid	293	513	12	11.	55.
2-amino-6-methylmercapto-purine	331	487	22	6.	29.

TABLE 5. (Continued)

- ^aAll molecules in 1M NaOH.
- ^bRange of linearity extends from detection limit to upper concentration where deviation from linearity is 1%. The ranges for the last five molecules is of order of 10 because of the rather high detection limits compared to the first four molecules (the high detection limits of the last five is a result of the high phosphorescence background at the respective excitation wavelengths).
- ^cLimit of detection (in $\mu\text{g/ml}$) is the concentration of analyte resulting in a signal-to-noise ratio of 2.
- ^dLimit of detection (in ng) is the amount of analyte resulting in a signal-to-noise ratio of 2; it is calculated from the concentrational value by using the sample volume of 5 μl .
- ^eThe luminescence of eosin Y at room temperature is a combination of E-type delayed fluorescence and phosphorescence.

Fig.6.--Room temperature phosphorescence analytical
curve for 4-amino-benzoic acid in 1M NaOH
absorbed on filter paper at 60°C.

Excitation spectrum - emission wavelength
set at 462 nm

Emission spectrum - excitation wavelength
set at 273 nm

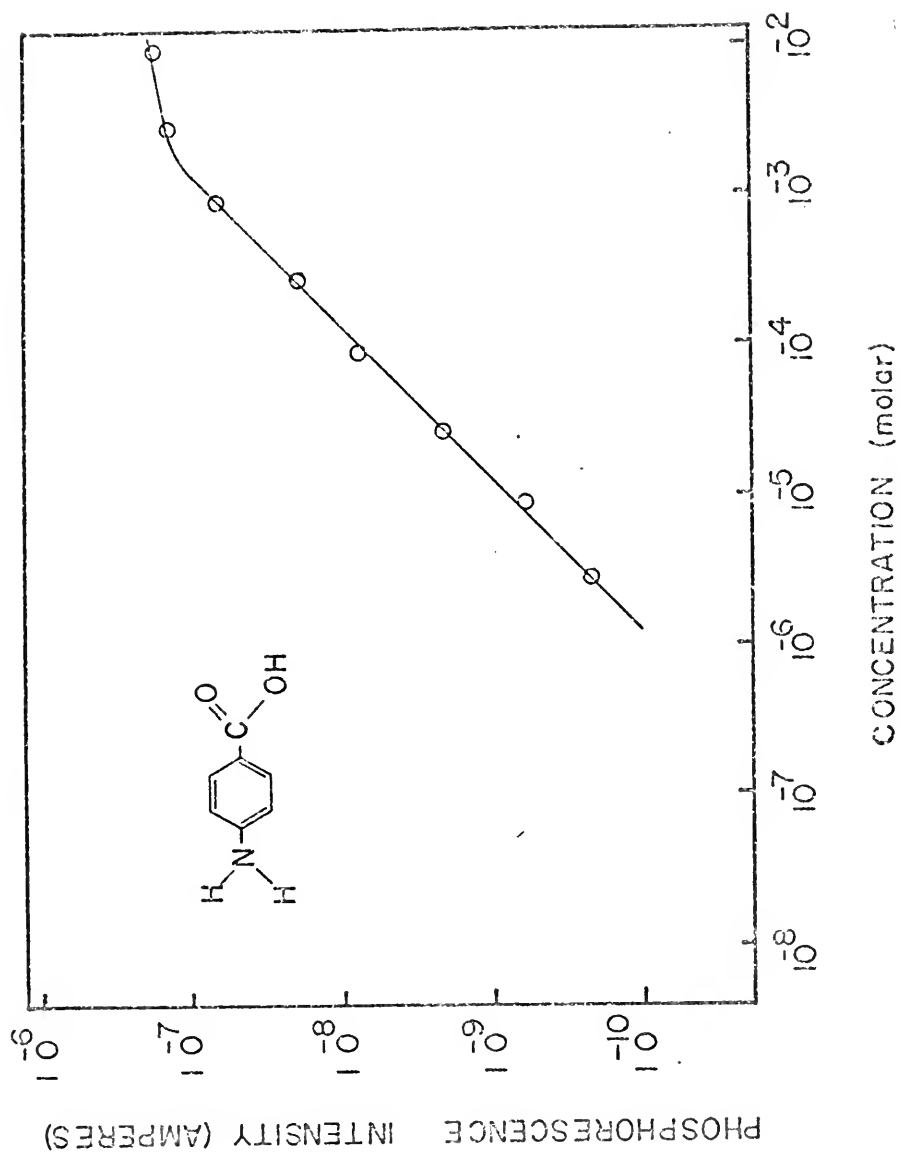


Fig. 7. --- Room temperature phosphorescence analytical curve for 4-amino-2,6-dihydroxyl-pyrimidine in 1M NaOH absorbed on filter paper at 60°C.

Excitation spectrum - emission wavelength
set at 421 nm

Emission spectrum - excitation wavelength
set at 310 nm

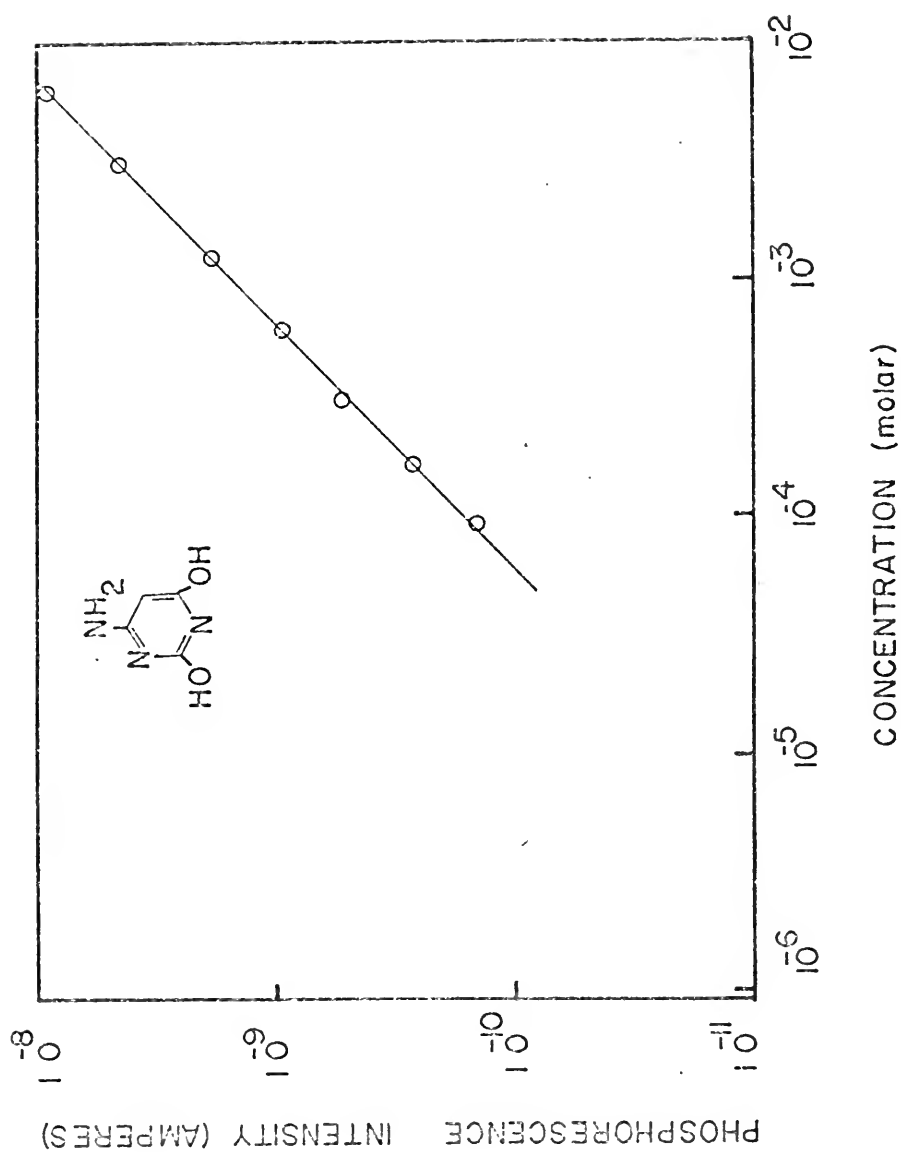


Fig. 8. --Room temperature phosphorescence analytical
curve for 2,6-diamino-purine in 1M NaOH
absorbed on filter paper at 60°C.

Excitation spectrum - emission wavelength
set at 451 nm

Emission spectrum - excitation wavelength
set at 286 nm

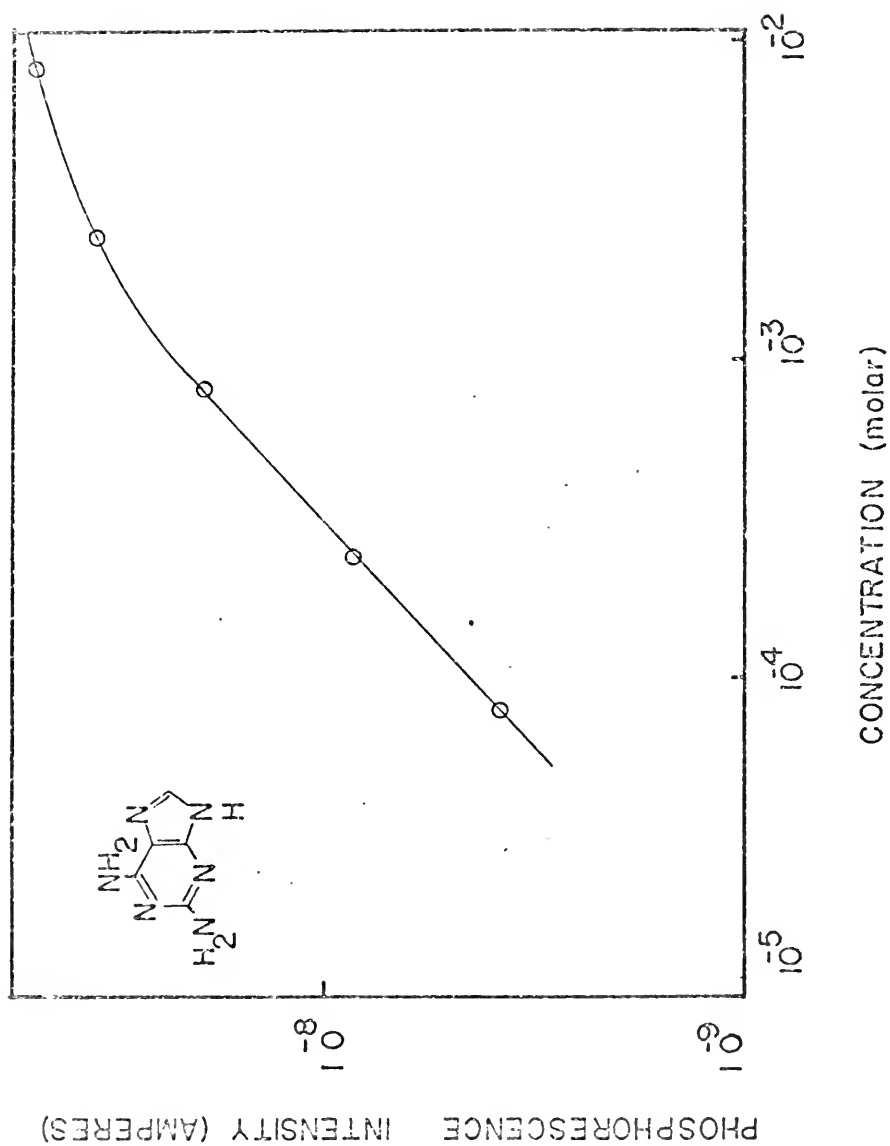


Fig. 9.--Room temperature phosphorescence analytical
curve for 6-methylmercapto-purine in 1M NaOH
absorbed on filter paper at 60°C.

Excitation spectrum - emission wavelength
set at 466 nm

Emission spectrum - excitation wavelength
set at 292 nm

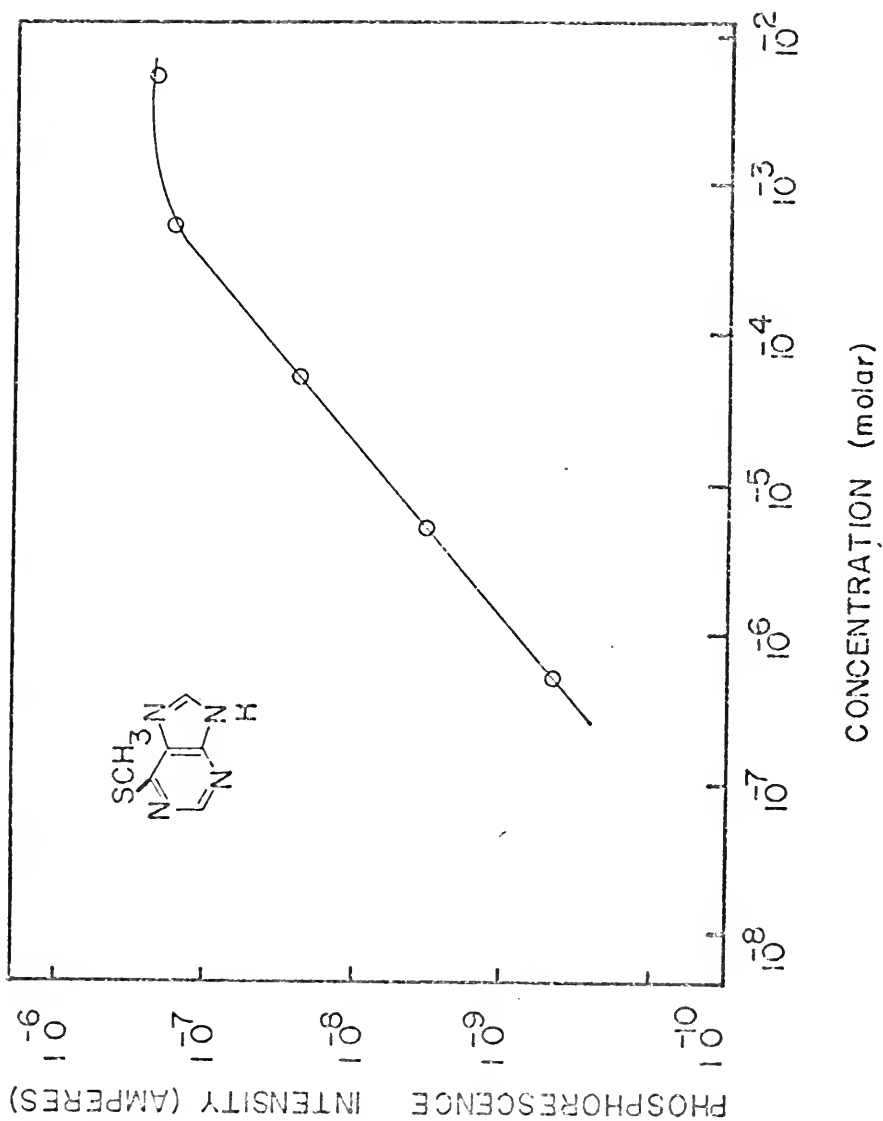


Fig.10.--Room temperature phosphorescence analytical
curve for sulfanilamide in 1M NaOH absorbed
on filter paper at 60°C.

Excitation spectrum - emission wavelength
set at 426 nm

Emission spectrum - excitation wavelength
set at 267 nm

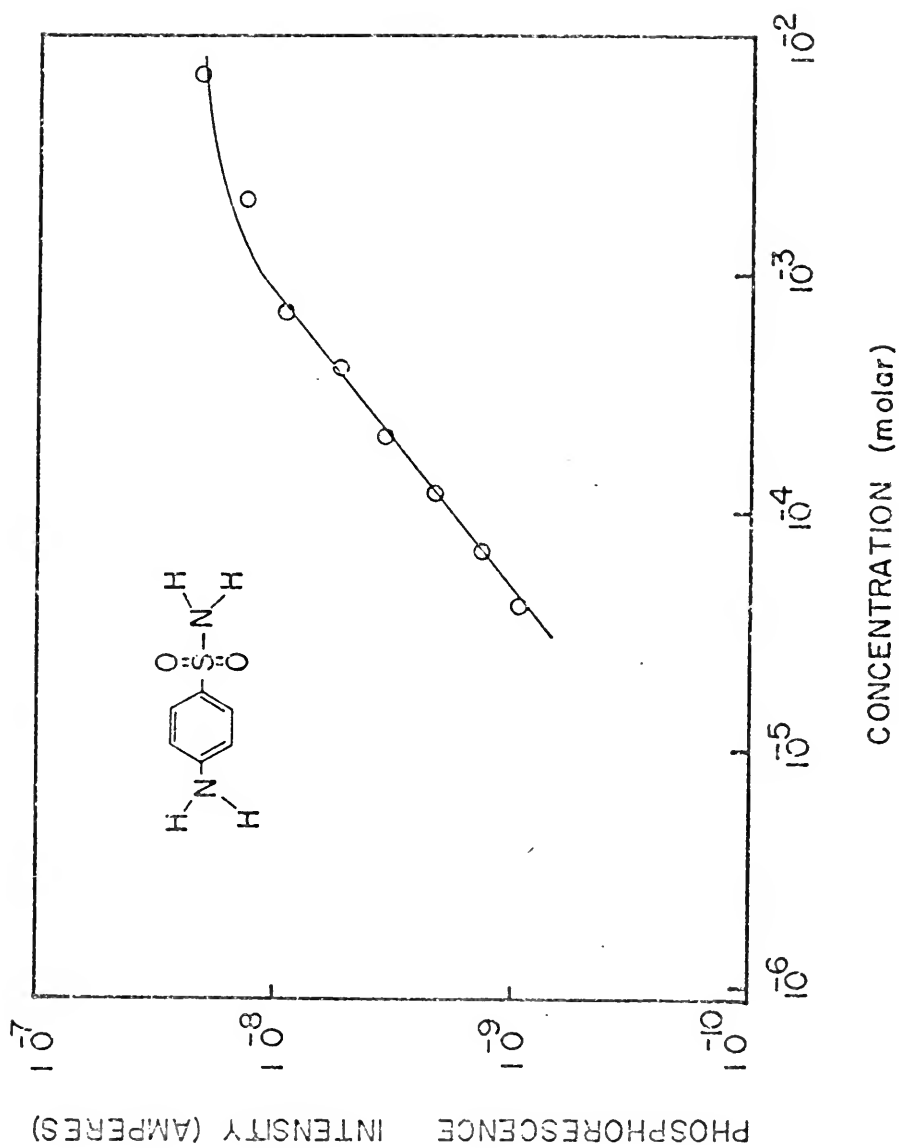


Fig.11.--Room temperature phosphorescence analytical
curve for sulfaguanidine in 1M NaOH absorbed
on filter paper at 60°C.

Excitation spectrum - emission wavelength
set at 426 nm

Emission spectrum - excitation wavelength
set at 267 nm

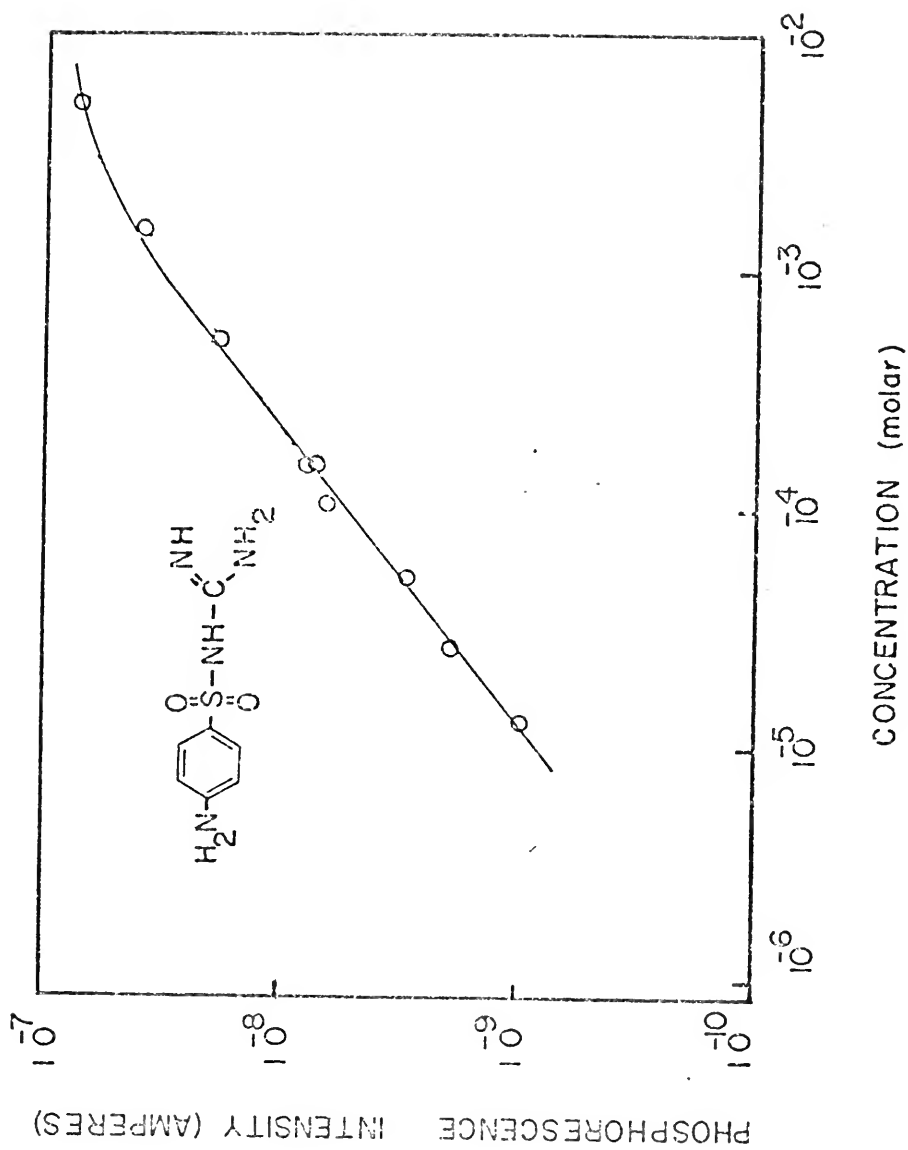


Fig.12.--Room temperature phosphorescence analytical curve for tryptophan in 1M NaOH absorbed on filter paper at 60°C.

Excitation spectrum - emission wavelength
set at 448 nm

Emission spectrum - excitation wavelength
set at 280 nm

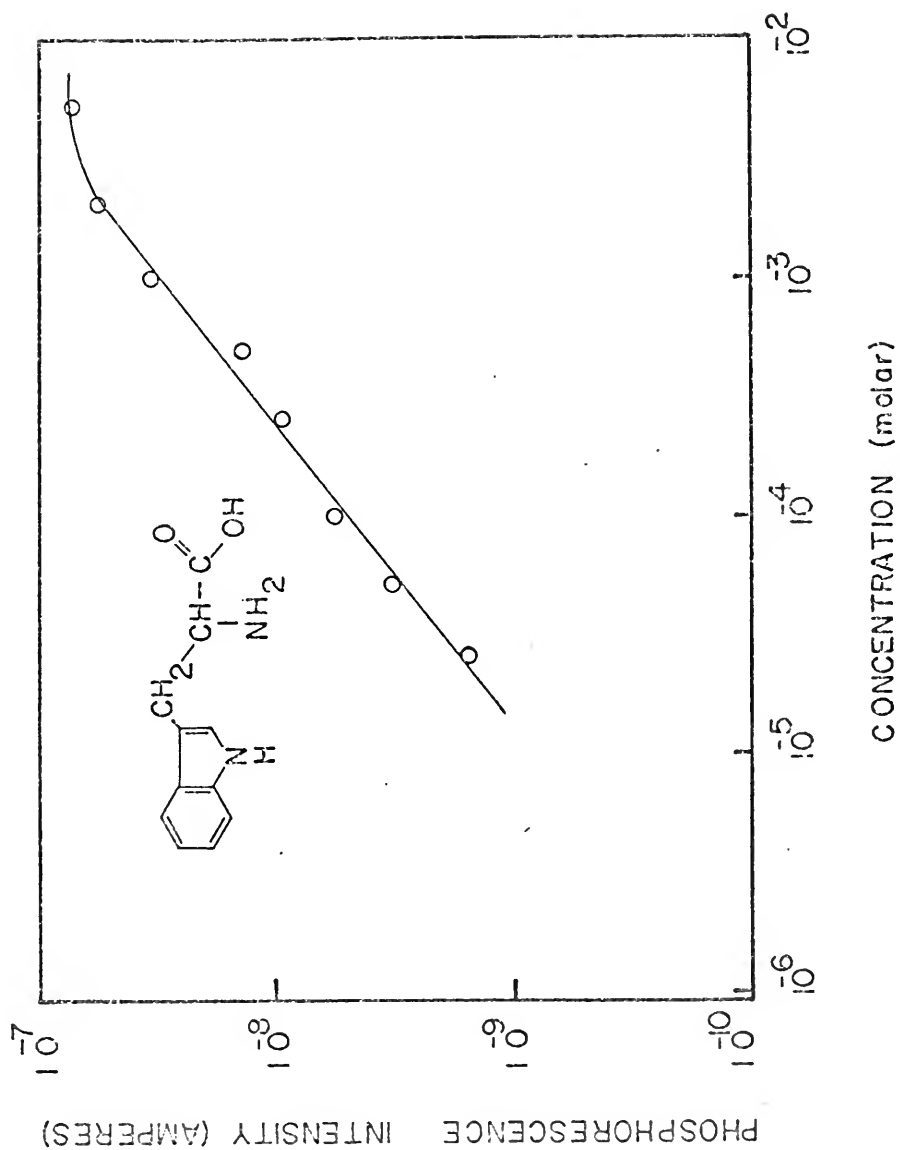


Fig.13.--Room temperature phosphorescence analytical
curve for 5-acetyl-uracil in 1M NaOH absorbed
on filter paper at 60°C.

Excitation spectrum - emission wavelength
set at 421 nm

Emission spectrum - excitation wavelength
set at 304 nm

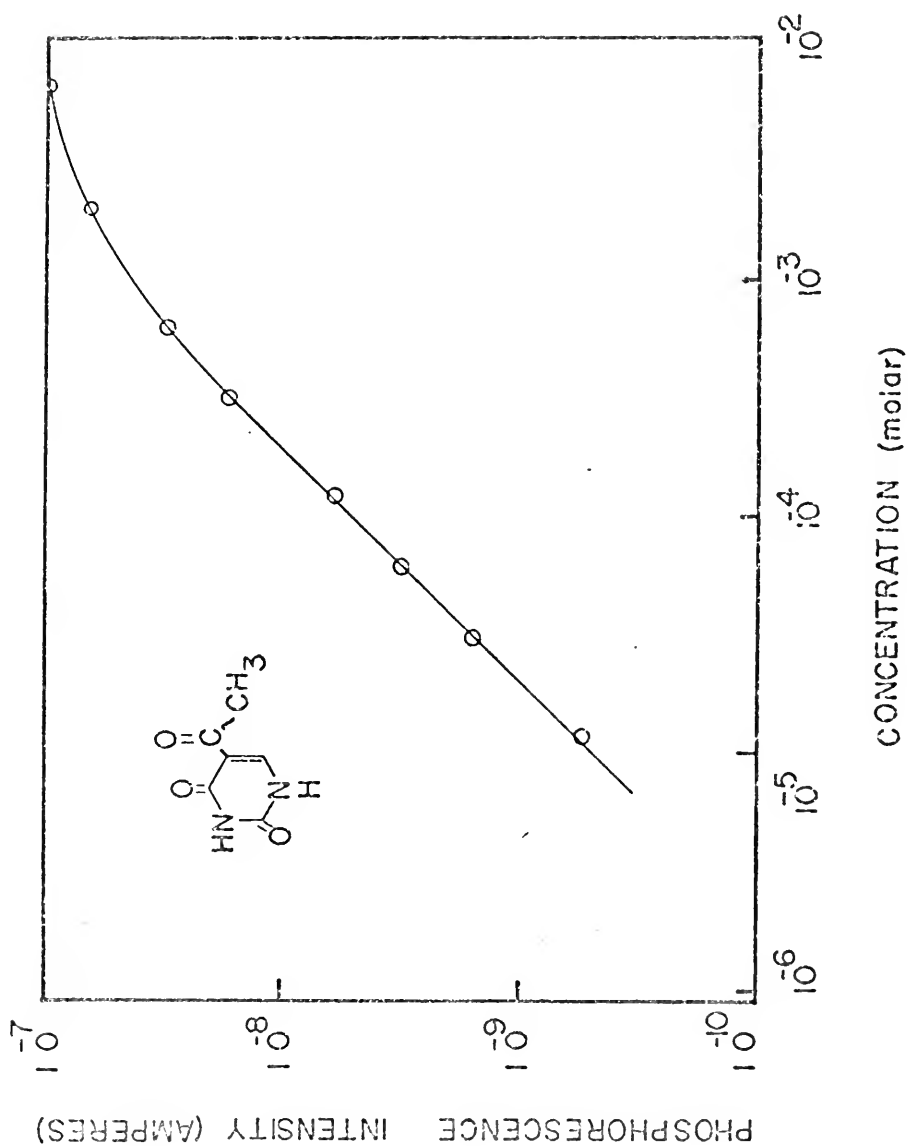


Fig.14.--Room temperature phosphorescence analytical
curve for 2-thio-6-amino-uracil in 1M NaOH
absorbed on filter paper at 60°C.

Excitation spectrum - emission wavelength
set at 441 nm

Emission spectrum - excitation wavelength
set at 307 nm

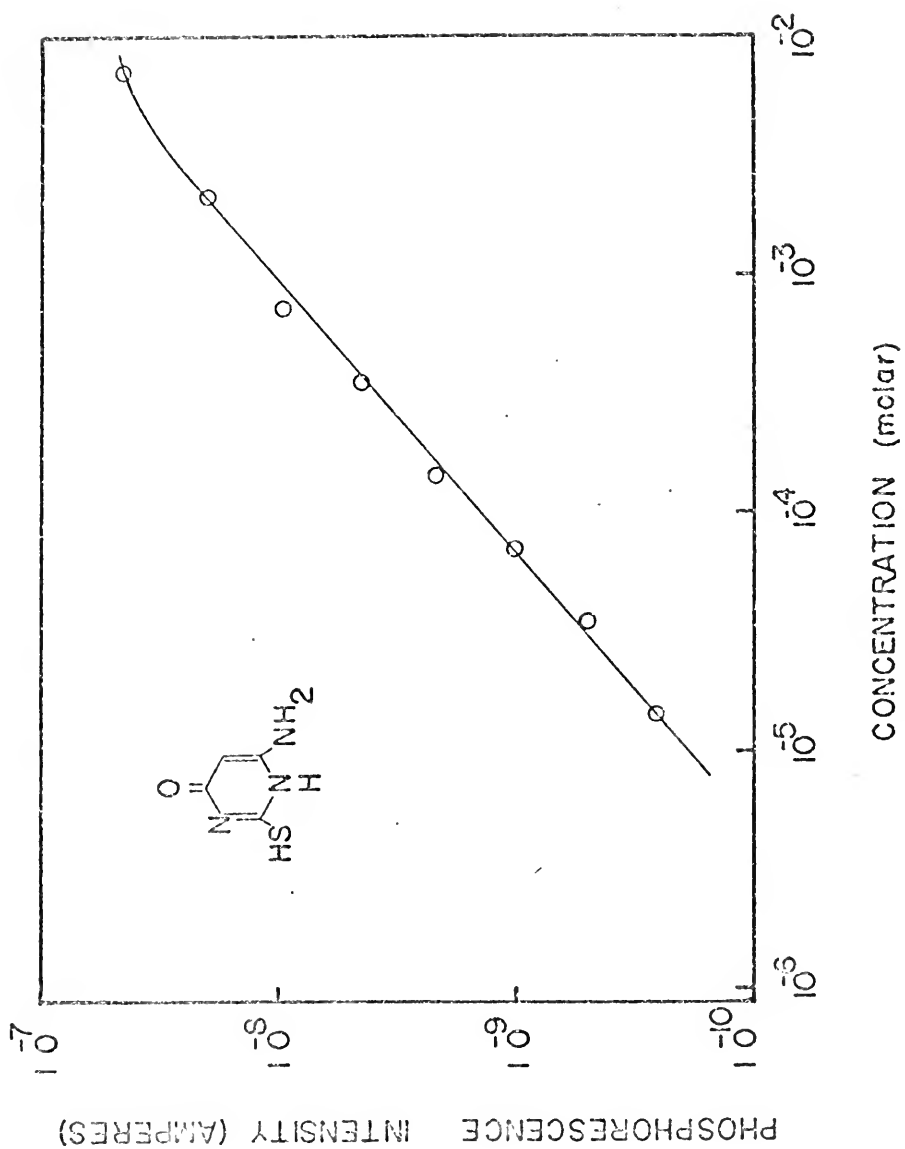
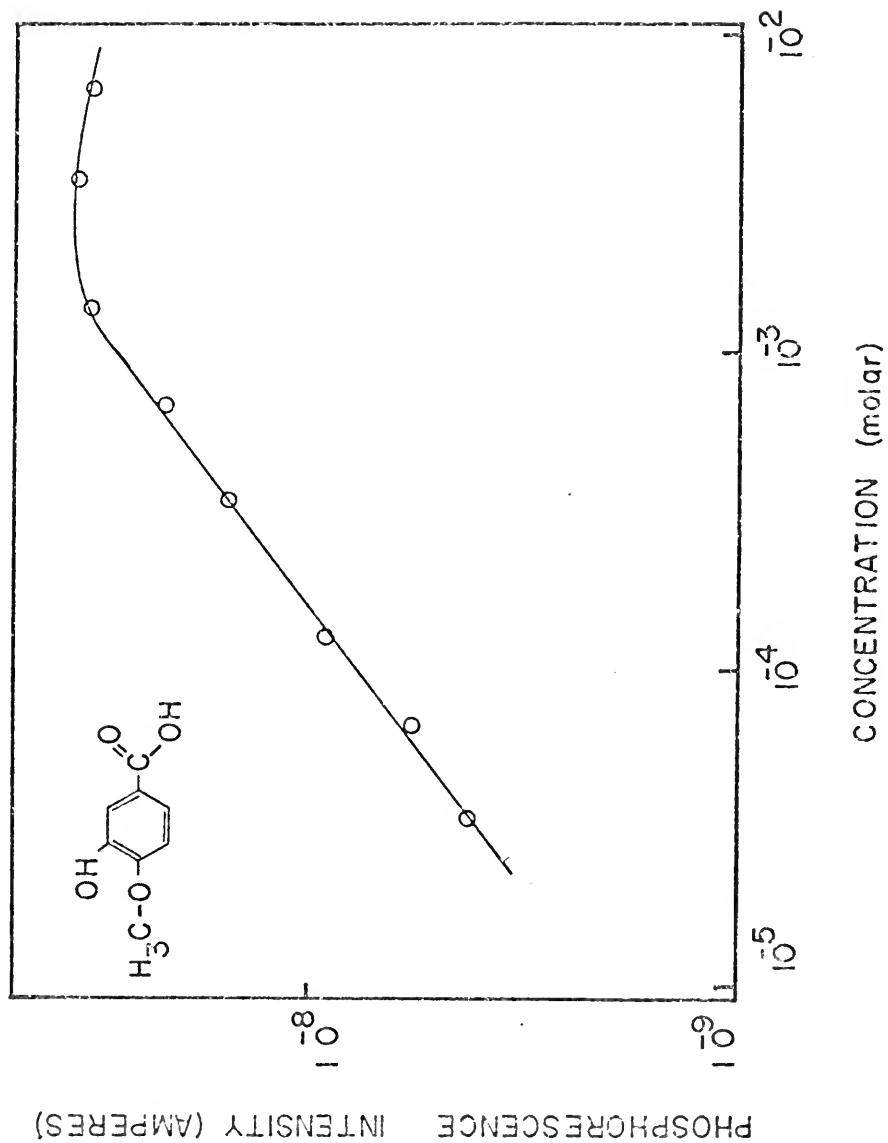


Fig.15.--Room temperature phosphorescence analytical curve for 4-hydroxy1-3-methoxy-benzaldehyde (vanillin) in 1M NaOH absorbed on filter paper at 60°C.

Excitation spectrum - emission wavelength
set at 510 nm

Emission spectrum - excitation wavelength
set at 332 nm



analytical curve was computed using a linear regression analysis program on the logarithms of the intensity and concentration values. The linear correlation coefficients for most of these plots were equal to or higher than 0.999. The limit of detection was determined to be the theoretical concentration for a signal-to-noise value of two. The percent relative standard deviation of the blank was calculated to be $\pm 5\%$ using a least squares error analysis.

Lifetime Effects

Lifetime measurements were attempted on a number of the room temperature phosphors. However, the nanometer had a limiting time constant of 0.05 sec. Also, using the Aminco-Bowman spectrophotofluorimeter, it is physically very difficult to measure lifetimes shorter than 0.1 sec accurately. Most of the compounds studied had phosphorescence lifetimes at room temperatures that appeared to be 0.1 sec or shorter and as a result, it was not possible to accurately measure them with this instrument. Schulman and Walling also examined the lifetimes of several molecules by flash photolysis and found them all to be in the msec range (16). Tryptophan,

which at 77°K had a decay lifetime of 6.3 sec was measured at room temperature to have a lifetime of 0.6 ± 0.1 sec. Most of these compounds listed in Tables 4 and 5 have lifetimes longer than 0.5 sec at low temperatures. Obviously, and as expected from theoretical considerations, higher temperatures greatly shorten phosphorescence lifetimes.

Spectral Effects

Another important result observed was a red shift in the phosphorescence emission spectra at room temperature as compared with 77°C. This effect was noted earlier (26). A shift of about 12 nm for tryptophan, and 20 nm for 6-methylmercapto-purine can be seen in Figures 16 and 17. Each set of spectra was produced from the same solution under identical experimental conditions. These shifts are not isolated cases as can be seen from a comparison of the values given in the Appendix and Table 4. For the family of purines studied, there was a red shift ranging from 20 to 40 nm between 77°C and room temperature phosphorescence emission maxima. Because the literature values may have been derived for different environmental situations, they should be

Fig.16.--Temperature effect on phosphorescence spectra of tryptophan (1mg/ml in 1M NaOH).

———— Room temperature ($\sim 330^{\circ}\text{K}$)
 ----- Liquid nitrogen ($\sim 77^{\circ}\text{K}$)

	Excitation Maximum	Emission Maximum
Room Temperature	290 nm	450 nm
Liquid Nitrogen	294 nm	438 nm

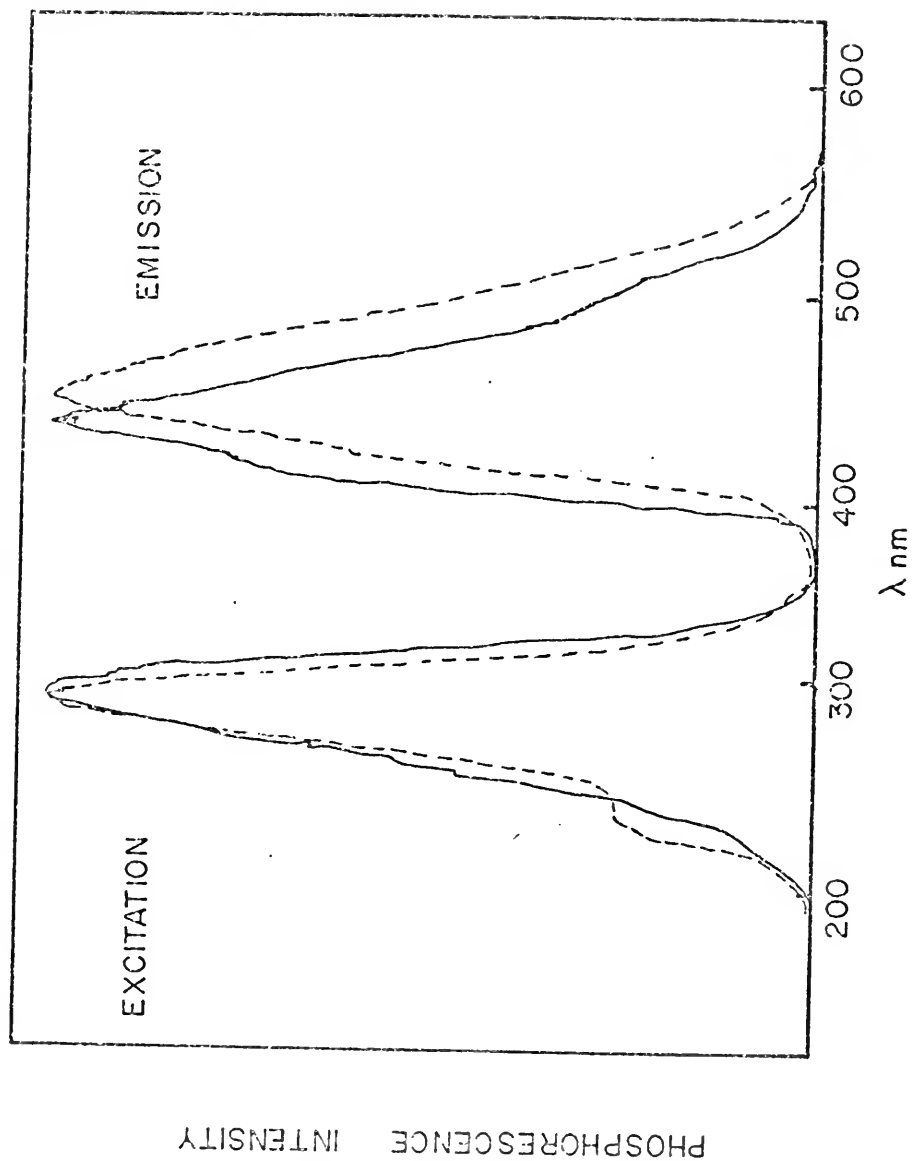
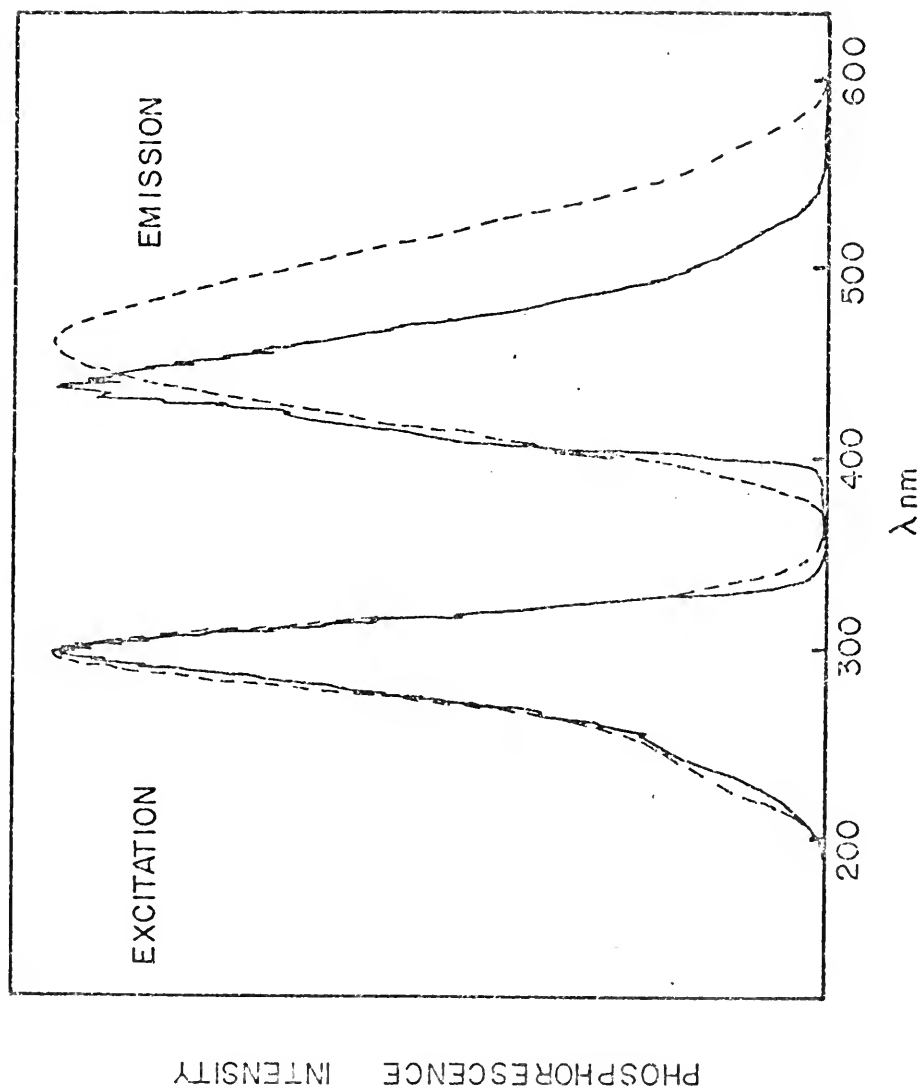


Fig.17.--Temperature effect on phosphorescence spectra of 6-methylmercapto purine (1mg/ml in 1M NaOH).

----- Room temperature (~ 330°K)
 ----- Liquid nitrogen (~ 77°K)

	Excitation Maximum	Emission Maximum
Room Temperature	298 nm	465 nm
Liquid Nitrogen	298 nm	445 nm



used with some caution. When more than one value was available, the one selected was nearest to the present conditions.

Comparison with Older Methods

The four analytical criteria mentioned earlier, i) breadth of application, ii) selectivity, iii) sensitivity, and iv) precision and accuracy, will now be considered in order to evaluate this method of analysis. Because room temperature phosphorimetry is in a very early stage of development, consideration should be given to its potential in addition to its present usefulness.

- (i) This study has shown that room temperature phosphorimetry, using aqueous 1M NaOH as the solvent and filter paper as the substrate, is applicable to a wide variety of compounds: acids, bases, phenols, etc. Also, those compounds that show E-type delayed fluorescence, e.g., eosin Y, can be measured with this technique. At present, the area of application is limited to those compounds that form stable ions under these experimental conditions. However, with further study, the potential exists that room temperature phosphorimetry could be extended to all those compounds

known to show strong phosphorescence at low temperatures or delayed fluorescence at room temperatures.

- (ii) Room temperature phosphorimetry offers good selectivity. As is the case in low temperature phosphorimetry or fluorimetry, substances can be differentiated using both their emission and excitation spectra. Further separation of phosphors is possible using time or frequency resolution techniques. Parameters, such as substrate, solvent, and pH can be more conveniently varied at room temperatures than at 77°K.
- (iii) Tables 4 and 5 show that this technique offers good sensitivity. The limit of detections extends from 20 ppb for eosin Y upwards to the µg/ml concentration region. Also, only a very small sample size of 5µl is needed. Conventional phosphorimetry requires a minimum of 20 µl and generally about 1 ml. Fluorimetry usually requires about 1 ml of solution. As a result, the absolute sensitivity of room temperature phosphorimetry is enhanced by a

factor of 200 over that of these conventional methods. An absolute limit of detection of 0.1 ng for 4-amino-benzoic acid was achieved using this technique. This compares favorably with the earlier results of Aaron and Winefordner. A fluorimetric detection limit of 10 ng and phosphorimetric detection limit of 1 ng for this compound was reported (27).

- (iv) The percent relative standard deviation of 5 %, two orders of magnitude above the detection limit, is quite good. It indicates that the use of filter paper and the present drying techniques are reproducible. It is also possible that the percent relative standard deviation can be reduced greatly with more sophisticated drying and handling techniques. Room temperature phosphorimetry, however, does not have some of the problems encountered at low temperatures. These include the variability in the cracking or snowing of the solid matrix, the noise from the wobbling capillary tube, or ice formation in the liquid nitrogen dewar. Thus, it is possible that greater precision could be

achieved than is possible at low temperatures. There is another important advantage that room temperature phosphorescence has as an analytical technique. It is a very fast technique; with the present drying procedure, samples can be handled at the rate of one sample per minute from the start of drying to the completion of measurement.

Rigidity Studies

The main purpose of this dissertation was to develop a new analytical technique using room temperature phosphorescence and to evaluate its usefulness. Another related and very interesting aspect would be the establishment of a strong theoretical basis for this phenomenon. Although no major attempt was made to solve this problem, several observations were made that are relevant to the discussion of the mechanism of room temperature phosphorescence.

A comparison of several of the analytes was made between their phosphorescence intensity when adsorbed on filter paper at room temperature and when frozen in a rigid matrix at 77°K. This comparison is given in Table 6.

TABLE 6. Comparison of Phosphorescence Intensities
at Room Temperature and at 77°K

Compound ^a	Max. Intensity at 77°K / Max. Intensity at 330°K
4-amino-benzoic acid	6
6-methylmercapto-purine	8
2-amino-6-methyl-mercapto-purine	19
4-amino-2,6-dihydroxyl pyrimidine	50
2,4-dithio-pyrimidine	1.8
sulfaquanidine	110
tryptophane	10
5-acetyl-uracil	220
2-thio-6-amino-uracil	26
vanillin	1.6

^aAll compounds prepared in 1M NaOH. Concentrations are approximately 5 mM.

Both intensities were measured under the same experimental conditions, with exception of the sample holder. The room temperature phosphorescence was measured using the apparatus described in Chapter III. The low temperature phosphorescence was measured using a 6 mm O.D. 1 mm I.D. rotating quartz capillary suspended in a quartz dewar filled with liquid nitrogen described by Winefordner (8). Therefore, the relative intensity ratios in Table 6 are indicative of the degree of rigidity with which the molecule is being held on the filter paper.

A trend that is apparent in this comparison is that the molecules that have the most ionic sites show the greatest rigidity. Vanillin and 2,4-dithio-pyrimidine are doubly charged in strong alkaline solution. On the other hand, compounds like sulfaquanidine and 5-acetyluracil which are expected to be the least ionized in strong base, show the greatest decrease in their relative phosphorescence intensities at room temperature. It should be noted that because both of these compounds exhibit strong phosphorescence ($S/N \sim 100$ at $10 \mu\text{g/ml}$) in alkaline solution, binding forces other than ionic (perhaps hydrogen bonding), may be involved in maintaining the required rigidity.

CHAPTER V

FUTURE STUDY

General

As is the experience with the development of any new technique of new device, there have been, in this study, both successes and some disappointments.

The areas of success include the development of a versatile technique that is rapid and reproducible. Another success is the demonstration that room temperature phosphorimetry can reach low detection limits.

Primary among the disappointments was the failure to reduce the phosphorescence background of the blank.

Substrate Modifications

Future study in room temperature phosphorimetry will make significant advances if substrate background can be reduced. A reduction of background noise of two orders of magnitude could easily be made before scattered light or instrumental noise would become the limiting factor. Such a reduction would result in the achievement of unusually low detection limits for many of the compounds studied.

Filter paper is made up of purified α -cellulose fibers. α -cellulose is a high molecular weight (50,000 to 500,000) polysaccharide composed of long straight chains of β -D-glucose arranged in $\beta(1\rightarrow4)$ linkages. There are three free alcoholic hydroxyl groups per sugar unit in a single chain of cellulose. Cellulose fibers are organized in bundles of parallel chains, cross-linked by hydrogen bonding (28,29). Neither D-glucose nor pure cellulose have an ultraviolet absorption spectra or give fluorescence or phosphorescence (30). Thus, the phosphorescence background must be coming from some by-product or residue left from the processing of the wood chips. The problem of reducing blank background can be approached from several different angles. One approach is further chemical treatment of the filter paper to remove, destroy, or mask the impurity. Another is to find some other cellulose product with no or little background. A third is to use something else besides paper for a substrate. A major advantage of filter paper is its superior handling qualities, inertness to strong alkali, and convenience. Other substrates might include silica gel, alumina, cotton fibers, and glass fibers.

Clinical Applications

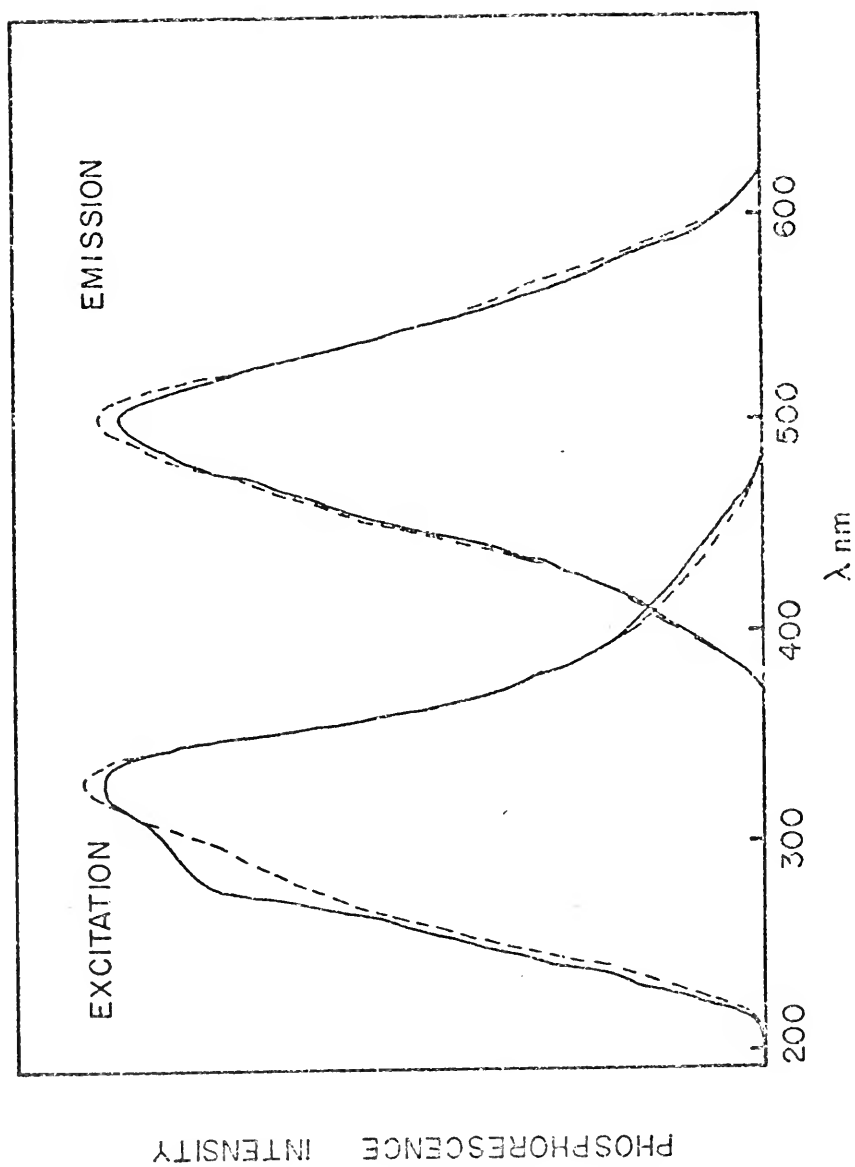
New methods of analysis should be attempted on urine or blood serum using the drugs and metabolites already shown to give room temperature phosphorescence and others, in particular, amphetamines, barbituates, etc. Please note that in serum analysis, room temperature phosphorimetry offers a technique with almost no interference from normal blood. The comparison of serum phosphorescence and background is given in Figure 18. Thus, this method offers the potential of an easy, rapid, sensitive analysis with few or no pre-treatment steps.

Air Pollution Applications

Room temperature phosphorimetry appears to have real use in airborne organic particulate studies. Many organic compounds could be separated from the airborne particulates by thin-layer or paper chromatography and then determined by direct room temperature phosphorimetry. Thus, small volumes of polluted air could be analyzed quickly. Because of the carcinogenic potential of many of the air pollutants, e.g., aromatic amines, ring-carbonyl compounds, and aldehyde precursors, this application of room temperature phosphorimetry would be important.

Fig.18.--Room temperature phosphorescence spectra of
Hyland Normal Control Serum in 1M NaOH and
filter paper background.

———— Serum spectra (1 part serum: 9 parts 1M NaOH)
----- Filter paper spectra



Other Luminescence Studies

Because these are so many parameters involved in a study of this nature, it was impossible to study all of them or even to investigate thoroughly any given one.

One parameter that has not been investigated thus far is the use of non-aqueous solvents. Theoretically, if one changes the solvent, then the pH and ionization can be varied to a different range. In this way, one could conceivably change the strength of ionic bonding to the substrate and control the phosphorescence of the analyte and its interferences.

One of the principal reasons for pursuing this investigation was the belief that if phosphorimetry could be inexpensively automated, it would be more readily accepted by industry and clinical laboratories as a useful analytical technique. While an automated procedure was not developed, it should be obvious that it is a realistic possibility. Further development of an automatic sampling system could be adapted to the Aminco spectrophotofluorimeter. The use of an enclosed drying chamber with controlled humidity should improve the sample precision.

A better understanding of the theoretical basis for

room temperature phosphorescence is another important area of future study. A theoretical understanding would allow systematic optimization of conditions to get the strongest bonding, greatest rigidity, and highest phosphorescence quantum yields.

As reported by Walling and Schulman (15, 16) and verified in this laboratory, phosphors which are thoroughly dried exhibit room temperature phosphorescence that is independent of oxygen quenching. At the present time, there is no reasonable mechanism explaining how ionic bonding or any other kind of bonding prevents oxygen molecules from deactivating the triplet state. Rigidity alone can not explain this anomaly satisfactorily. For example, phosphors embedded in solid polymer glasses gradually lose their luminescence properties at room temperatures because molecular oxygen permeates the polymer to quench the phosphorescence of the additive (31). Therefore, this particular aspect of room temperature phosphorescence should be considered a very important project for future study. The understanding of how oxygen quenching can be prevented could have application in the other luminescence techniques as well.

CHAPTER VI

SUMMARY

The present study indicates that room temperature phosphorescence has tremendous potential for analysis of a wide variety of ionic organic molecules and that it can be used for quantitative and qualitative identification purposes. The substances tested are representative of a wide variety of aromatic carboxylic acids, amines, thiols, or phenols. Many of the compounds are of biological importance or pharmaceutical interest.

The findings indicate that this method provides a sensitive, selective, and accurate means of identifying certain aromatic molecular species which are ionic. Although there is no strong theoretical explanation for this phenomenon, it is believed that the ionic state of the molecules results in great molecular rigidity via adsorption to the substrate, which reduces radiationless decay due to collisional deactivation.

Room temperature phosphorimetry offers many advantages as an analytical method. It has good sensitivity with the potential to be much more sensitive. It is inexpensive and safe in the sense that liquid nitrogen or cryogenic equipment is not required. It is convenient

and does not require exotic chemicals or high purity solvents. This technique is a rapid method of analysis which could be automated. The procedure established is fairly simple and would be easy for a technician to learn.

Room temperature phosphorescence definitely merits future study. The technique developed should find many applications to real systems, particularly for biological and pollution samples. Studies that are of importance for future research include the investigation of better substrates, automatic sampling, the influence of pH and solvent, and the analysis of species in blood serum, urine, and air pollution particles. Finally, it would seem that time-resolved phosphorimetry should provide additional selectivity in the measurement of real samples.

APPENDIX

SOURCES AND LOW TEMPERATURE PHOSPHORESCENCE CHARACTERISTICS OF ANALYTE MOLECULES USED IN ROOM TEMPERATURE PHOSPHORESCENCE STUDIES.

Compounds	Excitation Maximum, nm	Emission Maximum, nm	References
6-benzylamino-purine ^a	286	413	(32)
6-methyl-purine ^a	272	405	(32)
6-chloro-purine ^a	273	419	(32)
6-bromo-purine ^a	273	420	(32)
6-methylmercapto-purine ^a	298	445	
2,6-diamino-purine ^a	288	410	(32)
6-amino-purine ^a (adenine)	278	406	(32)
2-amino-6-methylmercapto purine ^a	321	456	(32)
2-amino-6-oxypurine ^a (guanine)	285	410	(33, 34)
uracil ^a	270	410	(35)
thymine ^a	270	430	(35)
cytosine ^a	270	400	(35)
2-amino-purimidine ^a	310	399	(36)
2,4-dithio-pyrimidine ^a	276, (322)	420	(36)
2-thio-6-amino-uracil ^a	285	412	(36)

Compounds	Excitation Maximum, nm	Emission Maximum, nm	References
4-amino-2,6-dihydroxyl- pyrimidine ^a	(255), 303	408	(36)
2-amino-4-methyl- pyrimidine ^a	w	w	(36)
2-amino-5-chloro-4- carboxylic acid- pyrimidine ^a	w	w	(36)
2-amino-4,6-dihydroxyl- pyrimidine ^a	w	w	(36)
4,5-diamino-uracil ^a	w	w	(36)
5-amino-uracil ^a	w	w	(36)
6-amino-uracil ^a	w	w	(36)
2-thio-4,6-dioxy- pyrimidine ^a	290	442	(36)
4,6-dihydroxyl-pyrimidine ^a	302	416	(36)
sulfanilamide ^a	276	409	(37, 38, 39)
sulfathiazole ^a	280	416	(39)
sulfamerazine ^a	280	405	(38)
sulfamethazine ^a	283	410	(38, 39)
sulfaguanidine ^a	273	410	(38)
sulfadiazine ^a	276	410	(38, 39)
pteroylglu amic acid (folic acid) ^a	300	505	(40)
riboflavin (vitamin B ₂) ^a	--	--	(40)
thiamine (vitamin B ₁) ^a	--	--	(40)

Compound	Excitation Maximum, nm	Emission Maximum, nm	References
calciferol (vitamin D ₂) ^a	--	--	(40)
vitamin K ₁ ^a	340	562	(41)
vitamin K ₃ ^a	340	545	(41)
vitamin K ₅ ^a	315	530	(41)
pyridoxine hydrochloride ^a (vitamin B ₆ HCl)	291	425	(40)
niacinamide ^a	270	410	(40)
4-amino-benzoic acid ^a	308	428	(40, 42, 43)
vanillin ^b	325	501	
epinephrine ^a	--	--	(44)
tyrosine ^a	280	388	
tryptophan ^b	295	440	(45, 46)
uridine ^a	270	420	(35)
thymidine ^a (2-deoxyribose)	270	435	(35)
cytidine ^a	--	--	(35)
adenosine ^a			
guanosine ^a			
norepinephrine ^a			
5-acetyl-uracil ^a			

^aSource: Nutritional Biochemicals Corp., Cleveland, Ohio 44101

^bSource: Eastman Chemicals, Kingsport, Tennessee 37662

^cprincipal maximum, shoulder in brackets.

w = weak

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BIOGRAPHICAL SKETCH

Stephen Lawrence Wellons was born in Franklin, Virginia on September 13, 1948. He graduated from Friendsville Academy in Friendsville, Tennessee in 1965 after completing three years of work in two years. He was valedictorian of his class.

He attended Guilford College and received his B.S. degree in chemistry, graduating with Departmental Honors in 1969. While at Guilford, he became a Dana Scholar and was a recipient of the Miles White Scholarship Fund as well as earning his Eagle Scout award.

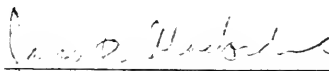
He entered the University of Florida as a Graduate School Fellow in the fall of 1969. As a conscientious objector, he worked for two years (1970-72) in the Department of Physiology at the University of Florida as a Laboratory Technologist II to fulfill the requirements of his alternative service. Since that time, he has been a graduate student in chemistry at the University of Florida working toward the degree of Doctor of Philosophy. While at the University of Florida, he has been employed as a graduate assistant.

He is a member of the American Chemical Society and Alpha Phi Omega, the national service fraternity. He is

also a private pilot with a commercial license and instrument rating.

He married Susan Ann Howard in December of 1972.

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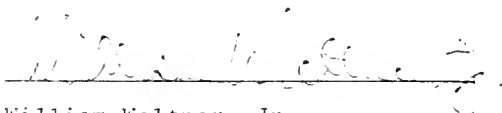
James D. Winefordner, Chairman
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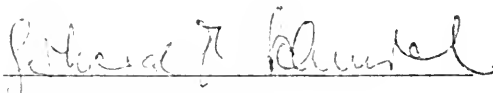
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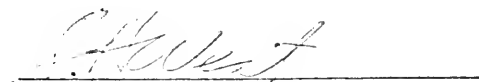
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Gerhard M. Schmid
Associate Professor of Chemistry

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Sherlie H. West
Professor and Assistant Dean for
Research

This dissertation was submitted to the Department of Chemistry in the College of Arts and Sciences and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

March, 1974

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